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Isolation of microorganism from acute diarrhea in children below 5 years and Comparison the inhibitory effect of natural honey and some types of antibiotics against bacteria isolated

¹Sura I. A. Jabuk, ²Raflaa S.H. Hussein , ³Hakim y. Rahi

⁴Zahraa M. Altaee and ⁵Hawraa M. R. ALRafyai

Affiliation: ^{1,2,4,5} University of Babylon, College of Science, Department of Biology, Babylon, Iraq.

³Babylon Educational Hospital for maternity and Children

Corresponding author: suraihsan@yahoo.com

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Abstract

The diarrhea is one of the most important disease infected children and lead them to enter the hospitals and increased vulnerability of our children to infection .In this study collect 25 stool samples from children the maternity and child hospital in Babil governorate by using clean and sterile cup . Then take samples to the microbiology laboratory to investigate the bacterial species causing diarrhea by cultured on the appropriate media and diagnostic biochemical tests were carried out. Present 16(36%) samples were positive obtained obtain from 25 samples belonging to the species of the following bacteria *klebsiella pneumonia*, *Enterobacter*, *staphylococcus aureus*, *Enteropathogenic*, *E.coli*, *pseudomonas aeruginosa*, *Proteus*. The effect of 10 types of antibiotic Cefixime, Amoxicillin Penicillin, Ceftriaxone, Erythromycin, Gentamycin, Streptomycin Trimethoprim + Sulfonamides, Nalidixic Acid, Imipenem was studied by using agar disc diffusion method . It was found that the Imipenem antibioticis most effective by affecting on all the bacterial isolated and least effective was Nalidixic acid. The most sensitive bacterial species were *Enterobacter sp.*, Which were sensitive to all antibiotics and the most resistant were the

proteus bacteria . When studying the effect of natural honey in inhibition of bacterial growth it was found to have a inhibition effect in preventing the growth of bacteria causing diarrhea similar the effect of antibiotic .

Keywords: : Diarrhea , Honey bee , Digestive system diseases

Introduction:

Diarrhea is the most common child disease. About 500 million children under the age of five years infected with diarrheal disease every year in the world. Diarrhea is one of the most common causes of child mortality in the world (5 million children a year) [1]. Diarrhea is increase in the amount and softness of the stool, so the stool is a fluid and incontinent and increases the number of defecations to reach 3 times or more per day. [2].

There are many factors that increase the chances of diarrhea in children such as : age of child under 6 months of age, malnutrition and vulnerability, contaminated food, polluted water and variability of seasons and dependence on artificial feeding increases the chances of diarrhea camper the children depend on breastfed[3].

Infection of the digestive system as a result of infection with various microorganisms such as viruses (rotavirus virus, adenoid virus) , bacteria (*E. coli*, *salmonella*, *shigella* and *Vibrio cholera*) , parasites (*Giardia lamblia* , *Entamoeba histolytica*) and other causes such as malnutrition, misuse of medicines and inflammation outside the digestive system. the honey bee is very useful for the prevention of diseases of the stomach and colon[4].

It has been proven that honey does not cost the digestive system effort to digest, because manganese and iron in honey helps digestion and representation of food, comparison industrial sugar, it leads to the digestive disorders increasing day by day, honey is preferred to all members of the digestive system in addition to the many benefits that Helps heal and fast treatment[5] . Honey has no adverse effect on the nervous system. It is made up of fructose, grape sugar and more than fifteen types of sugar. These sugars are easy to digest and the body does not need any complicated process to represent these sugars[6].

This study was aimed to isolated , identification and antibiotic sensitivity of bacteria cause diarrhea from children and study the effect of natural honey on inhibition bacterial growth.

Material and Methods:

- **Collection of samples**

Twenty-five samples of stool were collected from infants with diarrhea from both male and female , ranging in age from 1-5 years then transport quickly to the microbiology laboratory to identification of bacteria[7] . These samples were obtained from the maternity and children hospital in Babylon province.

- **Isolation and diagnosis**

Bacteria have been isolated from stool samples of children with diarrhea by Mix 1 g of stool samples with 1 ml of normal saline solution in sterile tube. Mixed vertically with Vortex and left vertically to allow the large particles to stagnate for 5

minutes at laboratory temperature, then the samples were cultured by spreading 0.1 ml of each samples on the cultured media (MacConky agar and blood agar) and incubated at 37 ° C for 24 h and diagnosis according to the[3]

- **The antibiotic sensitivity test**

Used nine type of antibiotics disc listed in Table (1) to examine the sensitivity of bacterial isolates from diarrhea ,by using the molar Hinton agar . Spreading 0.1 ml of 24-hour bacterial age by using a sterile spreader after comparing the turbidity of the bacterial growth with the normal turbidity standard (Macfarland standard) which gives an approximate number of cells 1.5x10⁸ cells per ml on the center of the Muller Hinton agar [8]. Then leave the plate for 5 minutes until the surface dry at room temperature, On the surface of the plant. The plates were incubated at 37 ° C for 24 h. The growth inhibitory zones were observed around the antibiotic disc , which were estimated at mm. The results were compared with the NCCLS (2007).

- **Use of honey as an antibiotic to inhibit the growth of isolated bacteria:**

The well method was used to investigate the susceptibility of honey to inhibit the growth of bacteria causing diarrhea . the natural honey add in the well by using Muller Hinton Agar cultured with isolated bacteria about 50 microliters and measured the area of inhibition after incubation for 24 hours at 37 ° C (7) [5].

Results and Discussion

The results obtained showed that 16 (36%) samples positive from the total 25 samples as shown in Table (1). These results are similar to those in Iran where 11 (31%) and higher than in Basrah 12 (13.2%) . This difference may be due to the number of samples, the time period for completing the research, the difference in the diagnostic methods used and the different environmental conditions of the sites of these studies[9].

The emergence of diarrhea in infants is due to several major causes, including artificial breastfeeding, incomplete integration of the immune system, pollution and lack of maternal health education. Diarrheal disease is a common disease in the world, especially in children's hospitals and health institutions that care for children, which is a serious epidemic diseases that lead to the deaths of millions of children every year[10].

Table (1) number and ratio of positive and negative samples

Age\Years	Positive samples NO.(%)	Negative samples NO.(%)	Total NO.(%)
1-2	6(24)	3(12)	9(36)
2-3	3(12)	1(4)	4(16)
3-4	4(16)	3(12)	7(28)
4-5	3(12)	2(8)	5(20)
Total	16(64)	9(36)	25(100)

The results obtained showed that the percentage of sample contain bacteria was 52% more than the percentage of the sample contain parasite only 16% from the total 25 samples as shown in Table (2).

Table (2) number and ratio of samples according to the type of infection

Type of infection	Number of sample	%
Bacterial infection	13	52
Parasite infection	4	16
Mixed infection	8	32
Total (%)	25	100

It was found that the enteropathogenic *E.coli* bacteria are the most common bacterial species, obtained 11 isolates belonging to this bacterial type in addition to the other bacterial species shown in Table (3).

This variation may be attributed to geographical location, varying environmental and climatic conditions, differences in the number of samples, the time period for research, as well as differences in the ages of children[11].

Table (3) the type and the number of bacteria isolated from diarrhea

Type of microorganisms	Number
Bacteria	
<i>klebsiella pneumoniae</i>	3
<i>Enterobacter</i>	5
<i>Proteus sp.</i>	2
<i>Staphylococcus aureus</i>	7
<i>Enteropathogenic E.coli</i>	11
<i>Pseudomonas aeruginosa</i>	2
Parasite	
<i>Giardia lamblia</i>	6
<i>Entameaba histolytica</i>	4

It was found that the Imipenem antibiotic is most effective by affecting on all the bacterial isolated and least effective was Nalidixic acid. The most sensitive bacterial species was *Enterobacter sp.*, Which were sensitive to all type of antibiotics and the most resistant was the *proteus* bacteria (Table 4).

Table (4)The inhibition zone of antibiotic (mm) against isolated bacteria

Bacteria \ Antibiotic	<i>k.pneumoniae</i>	<i>enterobacter</i>	<i>proteus</i>	<i>staph aureus</i>	<i>E.Coli</i>
Amoxacillin	R	S	S	S	S
Cefixime	R	S	R	R	R
Penicillin	R	S	R	S	S
Ceftriaxone	S	S	R	S	R
Erythromycin	R	S	S	R	S
Gentamycin	S	S	R	R	S
Trimethoprim + Sulfonamides	S	S	R	S	R-
streptomycin	S	S	S	R	R
Imipenem	S	S	S	S	S
Nalidixic acid	S	R	R	R	R

Intestinal family members, especially E. coli, are a good product of many beta-lactam enzymes. The resistance of gram-negative bacteria to penicillin and cephalosporin is due to their ability to produce beta-lactam enzymes that encode their production of cytosomal-derived genes. There is another type of enzyme called Extended Spectrum β -Lactamase (ES β Ls), which analyzes the beta-lactam ring[12].

It is clear from the above that the choice of antibiotic suitable for treatment is not random, but depends on the drug sensitivity tests on the isolated bacteria to find the appropriate antibiotic to eliminate them. In order to avoid the emergence of resistance and to obtain high efficiency in the treatment, it is necessary to choose an effective antibiotic against the infectious germ and determine its value and dose[13].

After testing the effect of honey by using the well method found the ability of honey to inhibition the growth of bacteria and as shown in the scheme (1). The results of this study are similar to the results of [7]. This may be due to the fact that the honey contains natural substances that are easy to digest and absorb, so it does not need conversion processes. In addition, honey contains yeast that helps the digestive system to complete its tasks[14].

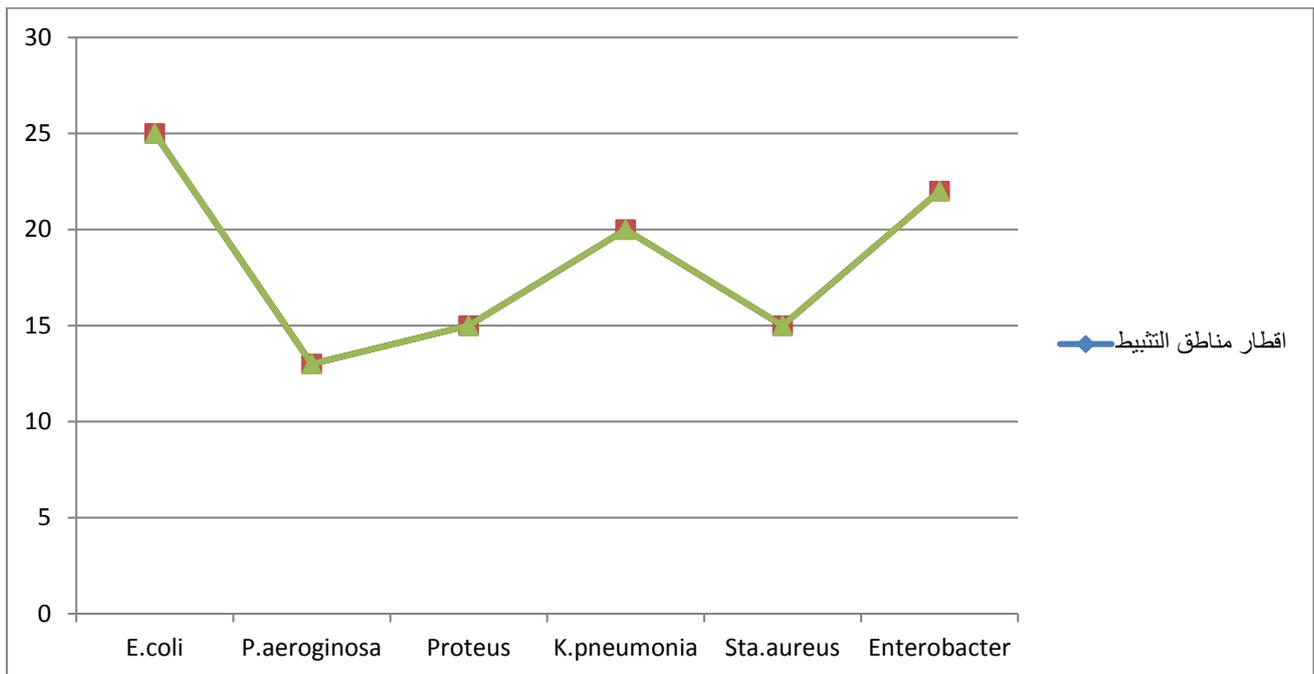


Fig.(1) The inhibition zone of natural honey (mm).

The effect of honey on the rest of the digestive system is very positive, has been recognized by many doctors that honey eliminates excess acidity in the stomach, so advise patients with ulcers or the twelve who complain of excess acidity in the stomach to be most honey or honey mixed with honey, It is recommended that in the case of stomach ulcers and duodenum, honey should be taken in a glass of warm water before eating at least two hours or three hours after supper, because dissolved honey in lukewarm water facilitates liquefaction[15].

Conclusions

The natural honey have a inhibition effect in preventing the growth of bacteria causing diarrhea similar the effect of antibiotic .

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