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Research Article

Synergism between bee honey and selected antibiotics against *shigella* species

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Abstract

Honey produced by the honey bees is one of the ancient traditional medicines used for treatment and prevention of various illnesses .In this study the antimicrobial effect of bee honey on organisms isolated from patients with acute Diarrhea in comparison with commonly used antibiotics for the treatment of infection was evaluated . **Objective :** One hundred and fifty five stool samples were collected from patients their age range from (6-45) years who were examined and diagnosed by specialist physicians as suspected with acute diarrhea during the period from May 2014 until end of November 2014 . **Methodology**: The stool samples were collected in sterile universal plastic containers and diagnosed according to well-known established bacteriological methods and then inoculated in to three enrichment media: Tetrathionate, Mannitol Selenite and Gram negative broth media , Subcultures have been made on Xylose-lysine-deoxycholate agar , Salmonella-Shigella agar (SS) and MacConkey agar media and incubated for 24 hrs at 37°C. Five antibiotic discs were selected (oxoid), including Ciprofloxacin (CIP) , Amoxicillin clavulinic acid (AMC) , and Ceftriaxone (CRO), Amikacin (AK) and Azithromycin (AZT) .The isolated pathogens were inoculated on Muller-Hinton agar, the susceptibility of the isolated organisms to honey , antibiotics and combination of both was studied .The mean inhibition zone produced by combination honey with antibiotics when applied to isolated *shigella* species was significantly higher than used of the antibiotic or honey alone. **Results:** *shigella* species were identified in 45 (29.03%) patients , in our study the number of males was higher than females(64%) , from 45(29.03%) *shigella species* isolated ,20(36%) were *shigella flexneri*, the most common isolate , *shigella sonnei* being the next most common isolate 12(21.8) followed by *Shigella dysenteriae* 9(16.3) and *shigella boydii* 4(7.2). **Conclusions:** Honey is famous rediscovered remedy which is cheap and nontoxic .It showed high inhibitory effect on the growth of *shigella species* and when used in combination with antibiotics it shows good synergistic effect on resistant bacteria. However, Pharmacological standardization and clinical evaluation of the effect of honey is necessary before using it as preventive or curative measure.

Keywords: Synergism, bee honey, antibiotics, shigella species

Introduction

The use of traditional and herbal medicine to treat infection was practiced since the origin of mankind, and in the past it was probably the only available method to be used for that[1].

Various plants and their extracts have already been in use for the treatment requiring antimicrobial activity and one of the popular natural antimicrobial substances described in the ancient medicine is honey[2]. Honey is the natural sweet substance from nectar or bossom or from the secretions of the living parts or excretions of plants which the honey bees collect and store in the honey [3], it was widely used in traditional medicine but it's use in modern medicine is limited because of the lack of scientific support [4].

Among its several uses , honey is used for the treatment of many infections , and also used effectively as wound dressing including surgical wounds , burns , and skin ulcers . It has been reported

that honey speed up the growth of new tissues and so help to heal the wound, reduces pain and odour quickly[5].

It has both bactreiocidal and bacteriostatic effect against various types of gram positive and gram negative bacteria such as *Staphylococcus .aureus* , *pseudomonas aeruginosa*, *Escherichia .coli, Staphylococcus. pyogenus* and *Salmonella typhi* [6].

This antibacterial effect is dependent on the concentration of the honey used and the nature of the bacteria isolated [7] and it has been attributed to it's high osmotic effect ,high acidic mature (pH3.2 - 4.5), hydrogen pen oxide concentration and it's phyto chemical nature other constituents of honey include proteins ,carbohydrates , vitamins , amino acide, peroxide ,amylase , fatty acids , phenol , and other compounds [8 , 9] .

As it is well known that infection is the most serious complication of wounds and burns accounted for 50 – 60 % of deaths due to sepsis in these patients despite improvement in antimicrobial therapy.

Therefore this study was planned to evaluate the in vitro antimicrobial effect of bee honey on pathogenic bacteria isolated from patients with acute Diarrhea compared with the effect of commonly used antibiotics in the treatment of infections .

Materials and Methods

Honey sample was collected from the Bee Keeping unit at the college of Agriculture, University of Baghdad. The honey sample was first filtered with sterile mesh to remove debris and to check it's microbial purity it was cultured on blood agar plate and incubated overnight at36 – 37c^{o}, then honey discs were prepared by using dry sterile filter paper having similarthickness and size (6mm), to the antibiotic disc used and then stored at 2-8c^{o} until use.

Bacteria

A total (155) samples were obtained from patients with acute Diarrhea from Baghdad General Hospital ,The stool samples were collected in sterile universal plastic containers and diagnosed according to well-known established bacteriological methods (1) and then inoculated in to three enrichment media: Tetrathionate, Mannitol Selenite and Gram negative broth media , Subcultures have been made on Xylose-lysine-desoxycholate agar , Salmonella-Shigella agar (SS) and MacConkey agar media and incubated for 24 hrs at 37 °C, The suspected isolates were transferred to Nutrient agar for further characterization, and morphological and biochemical tests

Antibiotic

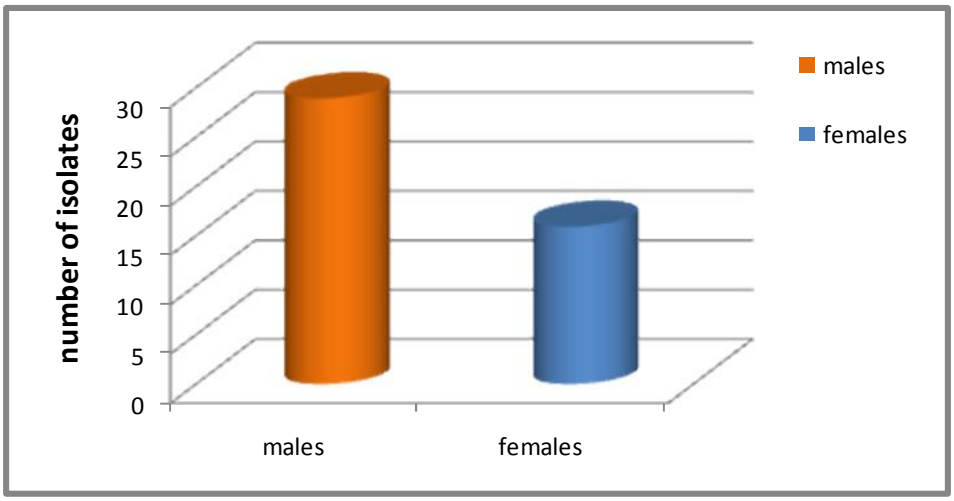
Five antibiotic discs were selected (oxoid), including Ciprofloxacin (CIP) , Amoxicillin clavulinic acid (AMC) , and Ceftriaxone (CRO), Amikacin (AK) and Azithromycin (AZT)The isolated pathogens were inoculated on Muller-Hinton agar. Each agar plate was divided by marker pen in to two halves , the antibiotic discs plated on one side and antibiotic discs immersed in honey was plated opposite the same antibiotic disc on the other side . At the center of the agar, disc immersed in honey was placed . Plates then incubated at 37c^{o} for 24hr and the diameter of the clear inhibition zone around each disc was measured and evaluated

Results and Discussion

During the study period , stool samples from (155) patients with acute Diarrhea, age range from 6-45 years tested by conventional methods .*shigella* species were identified in 45 (29.03%) patients , in our study the number of males was higher than females(64%) ,so the number of isolates was comparatively higher in males than females as shown in table (1) and figure (1) from 45(29.03%) *Shigella species* isolated ,20(36%) were *shigella flexneri*, the most common isolate , *shigella sonnei* being the next most common isolate 12(21.8) followed by *Shigella dysenteriae* 9(16.3) and *shigella boydii* 4(7.2) as shown in Table(2)

Table(1)A comparison of the number of isolates in males and females

Gender	No. of (+ve) Specimen	
	45	%
Male	29	64%
Female	16	34%



Figure(1)A comparison of the number of isolates in males and females

Table(2) showed the percentage & number of isolated sample .

Isolate	Number	%
Shigella flexneri	20	36
Shigella sonnei	12	21.8
Shigella dysenteriae	9	16.3
Shigella boydii	4	7.2

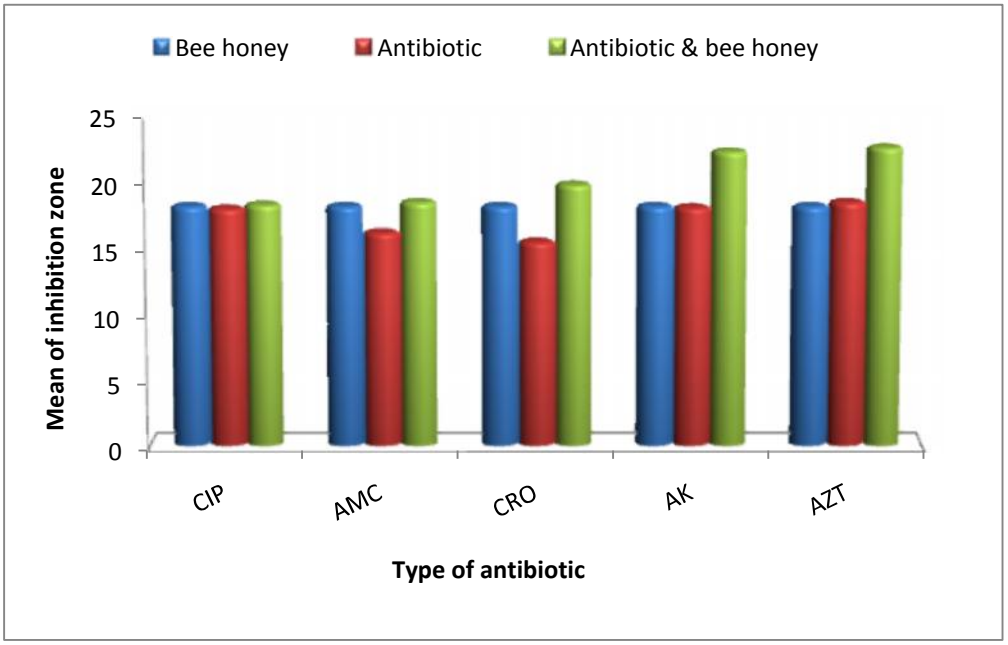
Effect of antibiotic, bee honey and combination of both on *shigella species* :

Table (3) and Figure (2) show demonstrate the effect of antibiotic, bee honey and combination of both on *shigella species*, Mean inhibition zone of the *shigella flexneri* were (18.0 ,16.2 , 15.5 , 20.1 , 20.5) for Ciprofloxacin, Amoxicillin / clavulinic acid, Ceftriaxone, Amikacin and Azithromycin Respectively, while the inhibition zone of the honey were 18.2.

When honey was added to the antibiotic disc , there was significant increase in the mean inhibition zone in respect to honey for Azithromycin, Amikacin and for Ceftriaxone while there was no significant increase on that Ciprofloxacin & Amoxicillin /clavulinic acid.

Table (3) Effect of antibiotic, bee honey and combination of both on *shigella flexneri*

	Antibiotic	Bee honey	Antibiotic & bee honey
	Inhibition Zone in mm	Inhibition Zone in mm	Inhibition Zone in mm
CIP	**18.0**	**18.2**	**18.2**
AMC	**16.2**	**18.2**	**18.2**
CRO	**15.5**	**18.2**	**19.8**
AK	**20.1**	**18.2**	**22.3**
AZT	**20.5**	**18.2**	**22.6**



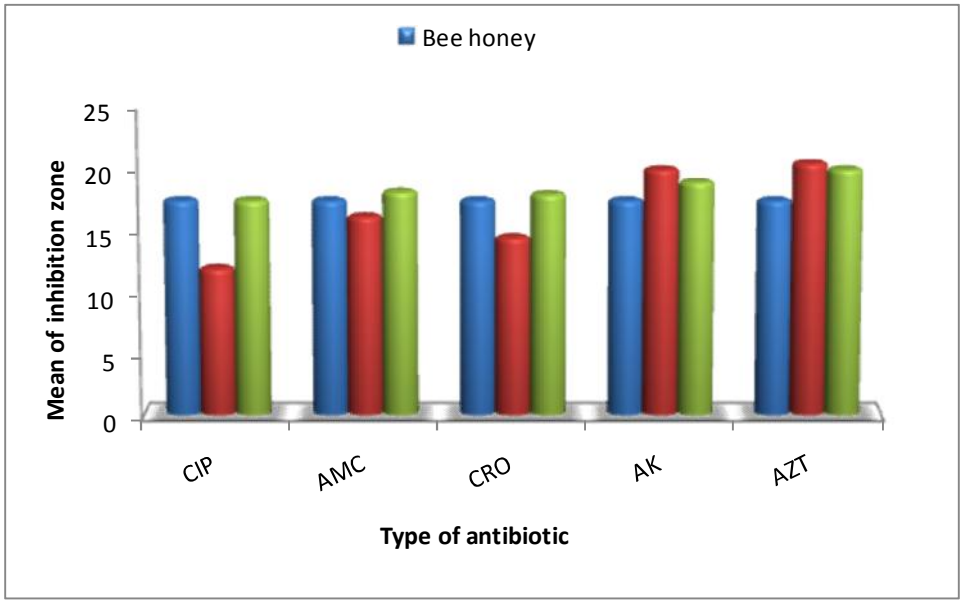
Figure(2). Mean inhibition zone of antibiotic, honey and combination of both on *shigella flexneri*

Table(4) and Figure(3) show demonstrate the effect of antibiotic, bee honey and combination of both on *shigella sonnei*, Mean inhibition zone of the *shigella sonnei* were (12.0 , 16.2 , 14.5 , 20.0 , 20.5) for Ciprofloxacin, Amoxicillin /clavulinic acid, Ceftriaxone, Amikacin and Azithromycin Respectively , while the inhibition zone of the honey were 17.5

When honey was added to the antibiotic disc , there was significant increase in the mean inhibition zone in respect to honey for Amoxicillin /clavulinic acid , Ceftriaxone, Amikacin and for Azithromycin while there was no significant increase on that Ciprofloxacin.

Table (4) Effect of antibiotic, bee honey and combination of both on *shigella sonnei*

	Antibiotic	Bee honey	Antibiotic & bee honey
	Inhibition Zone in mm	Inhibition Zone in mm	Inhibition Zone in mm
CIP	**12.0**	**17.5**	**17.5**
AMC	**16.2**	**17.5**	**18.2**
CRO	**14.5**	**17.5**	**18.0**
AK	**20.0**	**17.5**	**19.0**
AZT	**20.5**	**17.5**	**20.0**



Figure(3) Mean inhibition zone of antibiotic, honey and combination of both on *shigella sonnei*

Table (5) and Figure (4) show demonstrate the effect of antibiotic, bee honey and combination of both on *Shigella dysenteriae*, Mean inhibition zone of the *Shigella dysenteriae* were (15.0 , 15.2 , 17.5 , 21.0 , 21.0) for Ciprofloxacin, Amoxicillin /clavulinic acid, Ceftriaxone, Amikacin and Azithromycin Respectively , while the inhibition zone of the honey were 20.0 .

When honey was added to the antibiotic disc , there was significant increase in the mean inhibition zone in respect to honey for Amoxicillin /clavulinic acid , Ceftriaxone, Amikacin and for Azithromycin while there was no significant increase on that Ciprofloxacin .

Table (5) Effect of antibiotic, bee honey and combination of both on *Shigella dysenteriae*

	Antibiotic	Bee honey	Antibiotic & bee honey
	Inhibition Zone in mm	Inhibition Zone in mm	Inhibition Zone in mm
CIP	**15.0**	**20.0**	**20.0**
AMC	**15.2**	**20.0**	**22.2**
CRO	**17.5**	**20.0**	**22.0**
AK	**21.0**	**20.0**	**22.2**
AZT	**21.0**	**20.0**	**22.2**

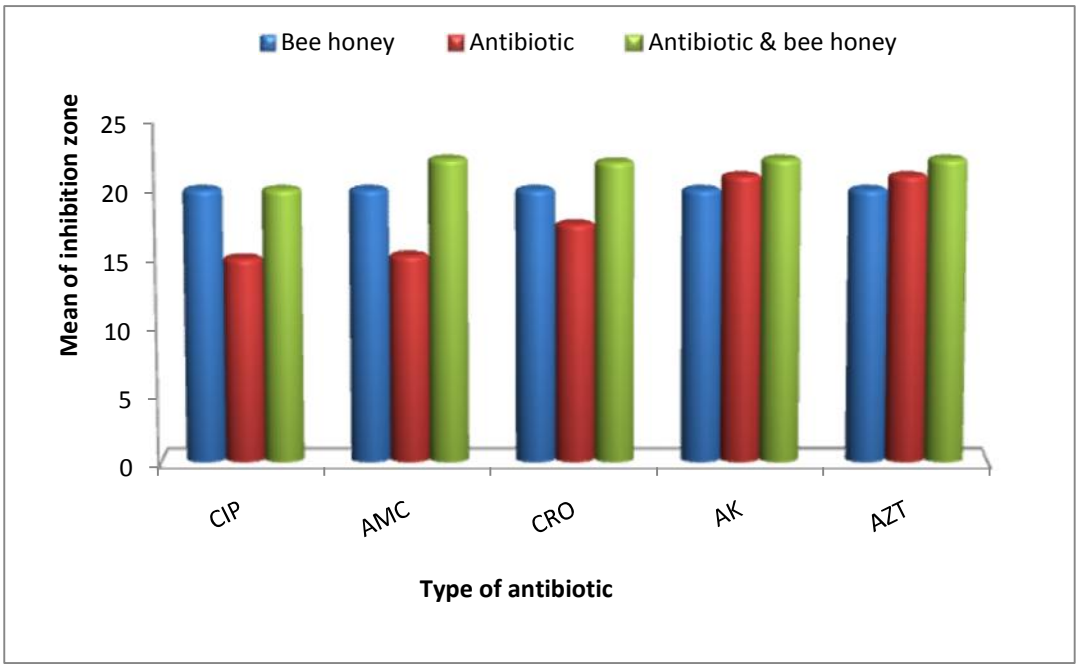


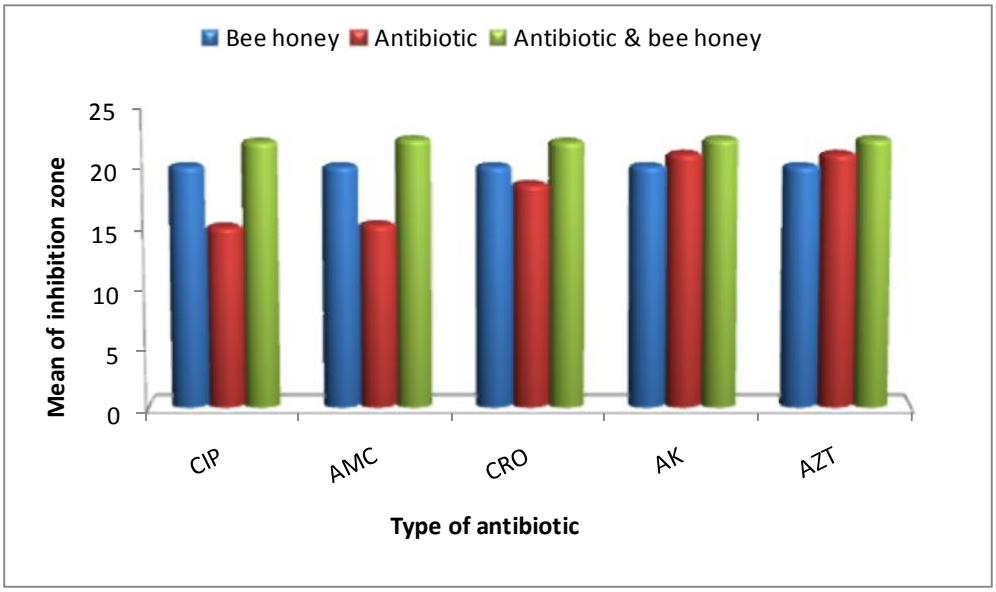
Figure (4) Mean inhibition zone of antibiotic, honey and combination of both on *Shigella dysenteriae*

Table (6) and Figure(5) show demonstrate the effect of antibiotic, bee honey and combination of both on *shigella boydii*, Mean inhibition zone of the *shigella boydii* were (15.0 , 15.2 , 18.5 , 21.0 , 21.0) for Ciprofloxacin, Amoxicillin /clavulinic acid, Ceftriaxone, Amikacin and Azithromycin Respectively , while the inhibition zone of the honey were 20.0 .

When honey was added to the antibiotic disc , there was significant increase in the mean inhibition zone in respect to honey for all antibiotics

Table (6)Effect of antibiotic, bee honey and combination of both on *shigella boydii*

	Antibiotic	Bee honey	Antibiotic & bee honey
	Inhibition Zone in mm	Inhibition Zone in mm	Inhibition Zone in mm
CIP	**15.0**	**20.0**	**22.0**
AMC	**15.2**	**20.0**	**22.2**
CRO	**18.5**	**20.0**	**22.0**
AK	**21.0**	**20.0**	**22.2**
AZT	**21.0**	**20.0**	**22.2**



Figure(5) Mean inhibition zone of antibiotic, honey and combination of both on *shigella boydii*

This study was undertaken to investigate in vitro antimicrobial activity of bee honey against *shigella species*. In the study, honey sample showed the antimicrobial activity, and our result were in agreement with Subrahmanyam *etal*(10) who found that honey inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas sp*., and also in agreement with Bilal *et al* (11) who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and Gram-positive bacteria,

Various researchers have shown that honey exerts an antibacterial activity against various organisms, including both gram-positive and gram-negative bacteria. The antibacterial activity of honey is mainly due to inhibiters in honey. These inhibiters are hydrogen peroxide, fiavinoids, and phenolic acids, plus many other unidentified inhibiters. A number of reasons for this have been suggested: shrinkage disruption of the bacterial cell wall due to the osmotic effect of the sugar content; induction of an unfavorable environment with low water activity (12,13)

Conclusion

Honey is famous rediscovered remedy which is cheap and nontoxic .It showed high inhibitory effect on the growth of *shigella species* and when used in combination with antibiotics it shows good synergistic effect on resistant bacteria. However, Pharmacological standardization and clinical evaluation of the effect of honey is necessary before using it as preventive or curative measure.

Recommendations

1. Effects of honey on multi-resistant *Staphylococcus aureus , E. coli* and *Salmonella typhi* .
2. Synergism between bee honey and other antibiotics.
3. Determine the minimum inhibitory concentration of bee honey on bacterial growth.
4. Study the effects of bee honey on some pathogenic fungi .

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