DOI: 10.24996/ijs.2018.59.1A.5

ISSN: 0067-2904

Study Antibacterial Activity of Honey Against Some Common Species of Pathogenic Bacteria

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Abstract

Honey is one of the most products has been used as an antimicrobial agent since the ancient times. The potential antimicrobial activity of honey is arise from its physiochemical proprieties such as high osmotic pressure, low pH, in addition to other factors and enzymes that work as antimicrobial agents. All these components and characteristics of honey led to consider it as one of the most efficient, useful and natural antimicrobial agents. From this point of view, recent study aim to evaluate the antibacterial activity of bee honey on some common species of pathogenic bacteria. The study included some of both gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus spp*.) and gram-negative bacteria (*Escherichia coli*, *Klebsiella pneunoniae*, and *Pseudomonas aeruginosa*). The antibacterial action of honey in three different concentrations (100%, 70% and 50%) was assessed. The results found that honey has the similar antibacterial effect as antibiotics effect. Additionally, the concentration of 100% was the most effective concentration in the inhibition of bacterial growth, and this concentration gave the best result of antibacterial effect. Furthermore, the results showed that as the concentration of honey decreased the effect of inhibition decrease as well. Finally, the study established that there is no significant different between the gram-positive and the gram-negative bacteria in the term of inhibition activity.

Keywords: honey, antibacterial activity, antimicrobial agents, antibiotic-resistant bacteria, gram-positive bacteria, gram-negative bacteria, pathogenic bacteria.

دراسة التأثير المثبط للعسل ضد بعض الأنواع الشائعة من البكتريا المرضية

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الخلاصة

العسل هو واحد من اكثر وافضل المواد التي استخدمت كمادة مضادة للماكروبات ومنذ القدم. ان التأثير التثبيطي العسل والمضاد للماكروبات ينشأ من كونة مادة غذائية طبيعية تتمتع بخصائص فيزيوكيميائية معينة تجعله مضاد جيد للكثير من الاحياء المجهرية ، ومن هذه الخصائص: الضغط الازموزي العالي ، الاس الهيدروجيني الواطئ، فضلا عن احتواءه على العديد من العوامل والانزيمات الاخرى التي تعمل كمواد مضادة للمكروبات. ومن هذا المنطلق جاءت هذه الدراسة والتي تهدف الى تقييم ودراسة التأثير التثبيطي للعسل المنتج من قبل النحل ضد بعض الانواع الشائعة من البكتريا المرضية. حيث شملت الدراسة كلا من البكتريا الموجبة والسالبة لصبغة غرام. استخدم العسل بثلاثة تراكيز مختلفة هي ١٠٠٪ و ٧٠٪ و ٥٠٪ . اظهرت النتائج ان

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للعسل تأثير تثبيطي مشابهه لتأثير المضادات الحيوية، وان تركيز ١٠٠٪ كان التركيز الاكثر فعالية في تثبيط النمو البكتيري مقارنة بالتراكيز الاخرى المستخدمة في الدراسة، وكلما قل تركيز العسل قلت الفعالية التثبيطية للعسل والعكس صحيح. واخيرا وليس اخرا، فقد اثبتت النتائج ان للعسل نفس الفعالية التثبيطة على كلا المجموعتين من البكتريا الموجبة والسالبة لصبغة غرام وانه لا يوجد اي فرق معنوي بين المجموعتين من الناحية الاحصائية.

Introduction

The over and inappropriate use of antibiotics led to the emergence and prevalence of antibiotic-resistant bacteria all over the world, which led to the high mordantly and morbidity among people and patients in the hospitals [1, 2].

This issue becomes one of the most problems attract high attention in the worldwide today. To solve this matter, alternative solutions should be taken such as, a new and novel antimicrobials agents like plants or other agents of plants sources [2- 4].

Honey is one of the most products has been used as an antimicrobial agent since the ancient times. Many studies reported that honey was used for handling of wounds and burns, as well as it has been used for treatment of sores, ulcers, gastritis, stomach and liver disease [5- 8].

Additionally, some times honey could be used in combination with certain herbal products for cure of mouth, throat, and cough especially in children with upper respiratory tract infections [9].

Honey is a sweet natural substance produced by the honeybees. The honeybees usually convert and process the nectar that collecting from the flowering plants to make the honey [2, 4, 7, 9].

The potential antimicrobial activity of honey is come from its physiochemical proprieties such as high osmotic pressure, low pH, low protein contains and high viscosity [7, 8, 10, 11].

Honey composed of about 181 substances, the great portion of these substances is sugars (38 % fructose, 31% glucose, 7.3% maltose, 1.3% sucrose), while the water contains present a bout 17%. The high osmotic pressure of honey (consider as antimicrobial factor) is due to the high concentration of sugar [7, 10].

Moreover, honey abundant of organic acids, approximately 30 different organic acids found in honey such as, gluconic acid, lactic acid, citric acid, succinic acid formic acid and acetic acid. The low pH of honey is attributed to the presence of these organic acids, and this characteristic play as inhibition factor for the growth of microorganisms [2].

Furthermore, honey has the ability to produce hydrogen peroxide (H_2O_2), which play essential role in the antimicrobial activity. The H_2O_2 of honey is produced as a result of breaking down of glucose by glucosidase enzyme [6, 7, 8, 9, 12].

Additionally, honey includes phytochemical factors such as, tetracycline derivatives, amylase, peroxides, phenols, fatty acids, ascorbic acid, terpenes, benzoic acid and benzyl alcohol, which play a vital role in antimicrobial activity of honey [4, 8, 9, 11, 12].

All these components and characteristics of honey led to believe that honey is one of the most efficient, useful and natural antimicrobial agents.Which it was used from the ancient times to nowadays to resolve many medical problems. From this perspective, this study aims to evaluate the antibacterial effect of bee honey against some common species of pathogenic bacteria. The study includes some of both gram-positive (*i.e. S. aureus, S. epidermidis* and *Streptococcus spp*.) and gram-negative bacteria (*i.e. E. coli*, *Klebsiella pneunoniae*, and *Pseudomonas aeruginosa*). Recent study was assessed the antibacterial activity of honey in three different concentrations (100%, 70% and 50%). Additionally, the comparison between the antibacterial activity of honey and some types of antibiotics were studied. Finally, the study will determine which groups of bacteria are more sensitive to the honey.

Materials and methods

Isolation and identification of Bacteria

About 20 bacterial isolates were collected from Baquba teaching hospital and Al-Muqdadia hospital, Diyala City, Iraq. The bacteria were isolated from different clinical specimens, including; stool, urine, sputum, nasal swabs, and throat swabs, burn and wound infections. The isolates were firstly cultured on the blood ager (Himedia- India), and incubated at 37°C for 24 hours. Then the isolated colonies were cultured on the MacConkey Agar, KingA media, *Pseudomonas* Selective

media, and Mannitol Salt Agar (Himedia-India). Finally, the well-isolated colonies were transferred to the Nutrient Agar (Himedia-India) for storage and biochemical tests.

The bacterial isolates were initially identified by colony morphology, Gram staining, and biochemical tests, which included Catalase test, Oxidase test, Coagulase test, EMB Agar test (Himedia- India), Urease test, Triple Sugar Iron (TSI) test (Himedia-India) and IMViC test [13, 14]. Finally, the isolates were identified by VITEK 2 COMPACT (BIOMERIEUX–France) for certain and definite identification of the bacteria. Accordingly, the results of identification were as in the Table-1.

The collection and preparation of Honey

The honey sample was collected from local market in sterile well-screwed container and kept in cool, dry and dark place in the laboratory. The honey was filtered with sterile mesh to remove any debris and storage until use. The honey solutions were prepared as the following: 5 ml of honey was mixed with 5ml of sterile distilled water to achieve a 50% (v/v) solution; another 7ml of honey was diluted with 3ml of distilled water to achieve a 70% (v/v) solution.

Antibiotic Susceptibility Test

The antibiotic sensitivity test was accomplished according to the Kirby-Bauer method [15]. Ten types of antibiotics disks were used which are; Amikacin, Amoxicillin/Clavulinic acid, Ceftriaxone, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Imipenem, Tetracycline and Vancomycin (Himedia- India). This test was performed to determine the susceptibility of each isolates to these antibiotics.

Antibacterial Activity of honey

The antibacterial activity of honey was tested by agar well diffusion method [6, 16, 17, 18]. The test was performed as the following: 4-5 colonies of the tested bacterial isolate were picked up from an overnight culture plate. Then the colonies emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No.0.5 turbidity standard. After that a sterile swab was dipped into the bacterial suspension, and the surface of a Mueller-Hinton agar plate (Himedia-India) was inoculated by bacterial isolate.

Finally, three wells were cut in the agar by a sterile cork borer of 6 mm diameter, the agar were removed with sterile needle. The three wells of each plate then filled with honey solutions; two wells were filled with 150µl of 50% (v/v) and 70% (v/v) solutions of honey, the third well was filled with 150µl of honey without any dilution (100 %). The plates were incubated at 37°C for 24 hours.

Statistical Analysis

The Data of the current study were analyzed by using (LSD) to compare between means of numeric data at level of significance of α=0.05 was applied to test. SPSS V.22 and Excel 2013 programs were used to analyze the data.

Results and discussion

The results of antibiotics susceptibility test indicated that the most bacterial isolates showed multidrug resistant pattern. In the case of gram-negative bacteria the results indicated that all bacterial isolates (100%) were resistant to the Amoxicillin/Clavulinic acid antibiotic. Furthermore, 90.9% of isolates were resistant to the Ceftriaxone. The resistance rates of the isolates to the Gentamicin and Ciprofloxacin were 45.4 %

On the other hand, the results showed that the most effective antibiotics were Amikacin and Imipenem, The results indicated that all gram negative isolates (100%) were sensitive to both of these antibiotics and the inhibition zones around these antibiotics disks were range between (16-30 mm) Figure-1.

In the case of gram positive bacterial isolates the results showed that all bacterial isolates (100%) were resistant to the both Erythromycin and Clindamycin antibiotics. Moreover, 77.7% of gram-positive isolates were resistant to the Amoxicillin/Clavulinic, Amikacin, Tetracycline, and Vancomycin. While, the resistance rates to both Gentamicin and Ceftriaxone were 66.6%.

Additionally, the results showed that the most effective antibiotics to the gram-positive isolates were Imipenem and Ciprofloxacin and the inhibition zones around these antibiotics disks were range between (21-41 mm) Figure-2.

Consequently, the results revealed that most of bacterial isolates in recent study were developed resistance to many types of antibiotics, which considered very common used antibiotics to treat many infection diseases.

So, these results suggested alternative solutions and treatments must be taken to resolve this problem such as other kinds of antibacterial reagents.

The results of well diffusion method found that honey inhibited the growth of all bacterial isolates that were used in this study (both gram positive and gram negative bacteria) and these results indicated that honey had a respectable antibacterial activity against many and different species of bacteria.

The results showed that the inhibition zone diameter of gram-negative bacteria around the wells that contain honey was range between (11-25 mm) Table-2, which was closer to the inhibition zone diameter around the antibiotics disks that bacteria are sensitive to them.

Also, the results of gram-positive bacteria showed that the inhibition zone diameter around the wells that contain honey was range between (6-27 mm) Table-3 which was still in the same range of sensitivity when compared with the results of antibiotics sensitivity test.

Moreover, the results indicated that the most effective concentration was 100% and the diameters of inhibition zone for bacteria around the wells of honey were decreased dramatically when the concentration of honey were decreased and vase reverse. Also, there was a significant different in the results among the three concentrations of honey that was used in this study, and this was true for both gram positive and gram-negative bacteria Tables-(2, and 3).

In the gram-negative bacteria, the diameter of inhibition zone was range between (18-25 mm) when honey's concentration was 100% (without dilution). While, the inhibition zone was range between (13-15 mm) when the concentration of honey was 70%. However, when the honey was used with the concentration of 50% the diameter of inhibition zone of bacteria around the well was range between (7-12 mm) (Table-2).

In the case of gram-positive bacteria the diameter of inhibition zone was ranged between (20-27 mm) when honey's concentration was 100% (without dilution). Whereas, the inhibition zone was range between (15-20 mm) when the concentration of honey was 70%. But, when the honey was used with the concentration of 50% the diameter of inhibition zone of bacteria around the well was range between (6-15mm) Table-3.

Additionally, the results showed that there is no significant different in the results of inhibition when we compared the two groups of bacteria; gram positive and gram negative Table-4. These results confirm that honey can be use as antibacterial agent against many species of bacteria.

Results of recent study are similar to the results of local study of Jawad (2011) [19], which showed that honey could inhibit the growth of gram positive bacteria (*S. aureus*) and gram negative bacteria (*Pseudomonas spp*.) and the inhibition zone significantly increased when the honey added to the antibiotic disks.

Similarly, the study of Mandal and Mandal (2011) [3] mentioned that the diameter of inhibition zones around the wells is about (13-14 mm) for *E. coli* bacteria and (0-20mm) for *S. typhimurium*, while it range between (15-16 mm) for *P. aeruginosa* , and (20-21mm) for *S. aureus.*

Additionally, Hussein *et al.* (2015) [20] evaluated the antibacterial activity of honey against gram positive and gram negative bacteria and they found that the most effective concentration was 40% or above.

In another study of Osho and Bello (2010) [21], comparison of the antibacterial activity of honey verses the antibacterial activity of antibiotics was made. They used honey in four concentration(5, 25, 50 and 100%) against *K. pneunoniae, P. aeruginosa, E. coli, S. aureus, and Bacillus subtilis.* They found that honey was most effective at 25% and 100%

Many other studies around the world indicated that honey has antibacterial activity against many types of pathogenic bacteria (both gram positive and gram negative bacteria). For instance: Taormina *et al.* (2001) [22] showed that honey of floral source has antibacterial effect against *E.coli, Shigella sonnei, S. thyphimurium, Listeria monocytogenes, S. aureus* and *Bacillus cereus* and the effect of inhibition depends on the concentration of honey that used in the test as well as on the tested bacteria.

Also, Washun and Kasa (2016) [4] tested the antibacterial activity of two types of honey (red and white honey) against many types of clinical bacteria such as *S. aureus*. They found that the red honey has a better antibacterial activity than white honey.

Furthermore, The study of Hegazi *et al.* (2017) [8] showed that honey inhibited the growth of five type of bacterial strain; *S. aureus S. mutans, K. pneumoniae, E. coli,* and *P. aeruginosa*. The results indicated that the antibacterial activity affected by pathogen and honey type.

Likewise, Shenoy *et al.* (2012) [1] revealed that honey could inhibit the growth of *P. aeruginosa* even when the honey was diluted with water many times and the bactericidal effect of honey at concentrations of 20%, 25%, 50% could killed the bacteria in 24 hours, but the higher concentrations 75% and 100% could killed the bacteria at 12 hours. This indicated that the high concentration of honey could be more effective than lower concentration when the duration of incubation decreased.

Molan (1992) [10] stated that there is many differences in the results of antibacterial activity of honey among different studies and this is because honey samples are different in there antibacterial ability and the species of bacteria can be different in there respond to the antibacterial factors that found in honey.

The antibacterial activity of honey is attributed to many factors and characteristics that found in honey such as high osmotic pressure, low pH, low protein contains in addition to the many other enzymes and factors such as; H_2O_2, lysozyme, organic acids, tetracycline derivatives, amylase, peroxides, phenols, fatty acids, ascorbic acid, terpenes, benzoic acid and benzyl alcohol, flavonoids, vitamins, catalase, glucose oxidase, ascorbic acid, carotenoid, antioxidant components. All these compounds play a vital role in antimicrobial activity of honey [4, 9, 10, 12].

In our study the greater value of inhibition found in the concentration of 100% and the inhibition rate was decreasing as the concentration of honey decrease (diluted of honey with water). This can be attributed to the osmotic effect of honey, because honey is a saturated solution of sugars and the water content is about 15-21% of all honey weight. This ratio of free water considered insufficient for growth of microorganism, this free water is called the water activity (a_w) the mean values of water activity of honey has reported to range between (0.5-0.6), while the growth of many species of bacteria could be inhibited by the a_w range (0.94-0.99). So the low a_w honey help in the inhibition the growth of many types of bacteria [2, 10].

The chemical profile of honey different among honey types and these differences depending upon the many factors such as: the floral source of the nectar, the year and time when was honey collected, and even the type of bee that producing the honey. All these factors affected the honey chemical compounds; as a result this will affected the potential activity of honey in the inhibition of bacteria [2]. Other factors that effect on the antibacterial activity of honey is the geographic location and environmental condition of the floral source, also the age and the health of bee colony could be affect the honey quality and its antibacterial activity [6].

Table 1- Illustrated the bacterial isolates that included in the study and their identification information by VITEK 2 COMPACT

No.	The isolates	Identification Information by VITEK 2
1	*E. coli*	99% Probability *E. coli*
2	*E. coli*	98% Probability *E. coli*
3	*E. coli*	99% Probability *E. coli*
4	*E. coli*	96% Probability *E. coli*
5	*E. coli*	99% Probability *E. coli*
6	*Klebsiella pneunoniae*	99% Probability *Klebsiella pneunoniae*
7	*Klebsiella pneunoniae*	93% Probability *Klebsiella pneunoniae*
8	*Klebsiella pneunoniae*	99% Probability *Klebsiella pneunoniae*
9	*pseudomonas aeruginosa*	99% Probability *pseudomonas aeruginosa*
10	*pseudomonas aeruginosa*	97% Probability *pseudomonas aeruginosa*
11	*pseudomonas aeruginosa*	99% Probability *pseudomonas aeruginosa*
12	*Staphylococcus aureus*	99% Probability *Staphylococcus aureus*
13	*Staphylococcus aureus*	97% Probability *Staphylococcus aureus*
14	*Staphylococcus aureus*	98% Probability *Staphylococcus aureus*
15	*Staphylococcus aureus*	99% Probability *Staphylococcus aureus*
16	*Staphylococcus epidermidis*	99% Probability *Staphylococcus epidermidis*
17	*Staphylococcus epidermidis*	94% Probability *Staphylococcus epidermidis*
18	*Staphylococcus epidermidis*	96% Probability *Staphylococcus epidermidis*
19	*Streptococcus pneumoniae*	99% Probability *Streptococcus pneumoniae*
20	*Streptococcus pneumoniae*	98% Probability *Streptococcus pneumoniae*

Table 2- The antibacterial activity of honey against Gram-Negative bacteria

Gram-Negative bacteria	Mean of Inhibition zone diameter (mm) of bacterial isolates when honey used at three different concentration			
	100 % (V/V)	70 % (V/V)	50% (V/V)	LSD
K. pneumoniae	18	13	7	6.52
E.coli	25	15	12	8.33
P. aeruginosa	22	15	11	6.63
LSD: Least Significant Difference				

Table 3- The antibacterial activity of honey against Gram-Positive bacteria

Gram-Positive bacteria	Mean of Inhibition zone diameter (mm) of bacterial isolates when honey used at three different concentration			
	100% (V/V)	70% (V/V)	50% (V/V)	LSD
S. aureus	23	20	15	4.21
S. epidermidis	20	15	6	3.25
S. pneumoniae	27	17	13	6.11
LSD: Least Significant Difference				

Table 4- Comparison between Gram-Negative and Gram-Positive Bacteria in the term of antibacterial activity of honey

Bacterial Group	Bacterial Isolates	Mean of Inhibition zone diameter (mm) when honey used at concentration			
		100% (V/V)	70% (V/V)	50% (V/V)	LSD
Gram-Negative bacteria	*K. pneumoniae*	18	13	7	NS
	E. coli	25	15	12	
	P. aerugenosa	22	15	11	
Gram-Positive bacteria	*S. aureus*	23	20	15	
	S. epidermidis	20	15	6	
	S. pneumoniae	27	17	13	
LSD: Least Significant Difference NS: Not Significant					

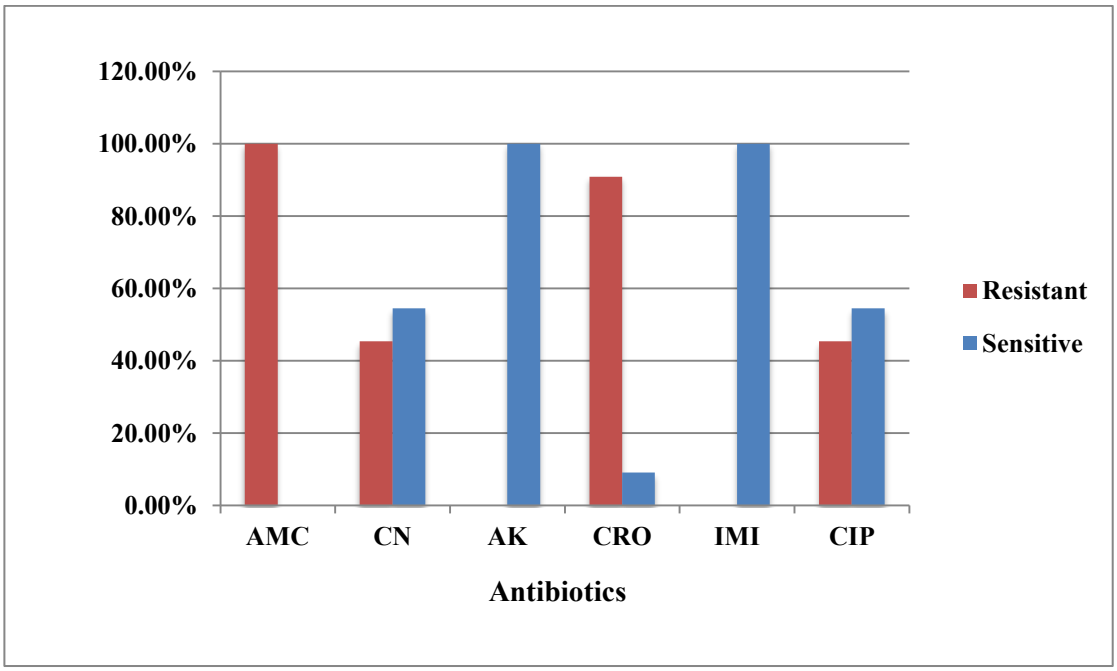

Figure 1- The results of antibiotic susceptibility test for Gram-Negative bacteria

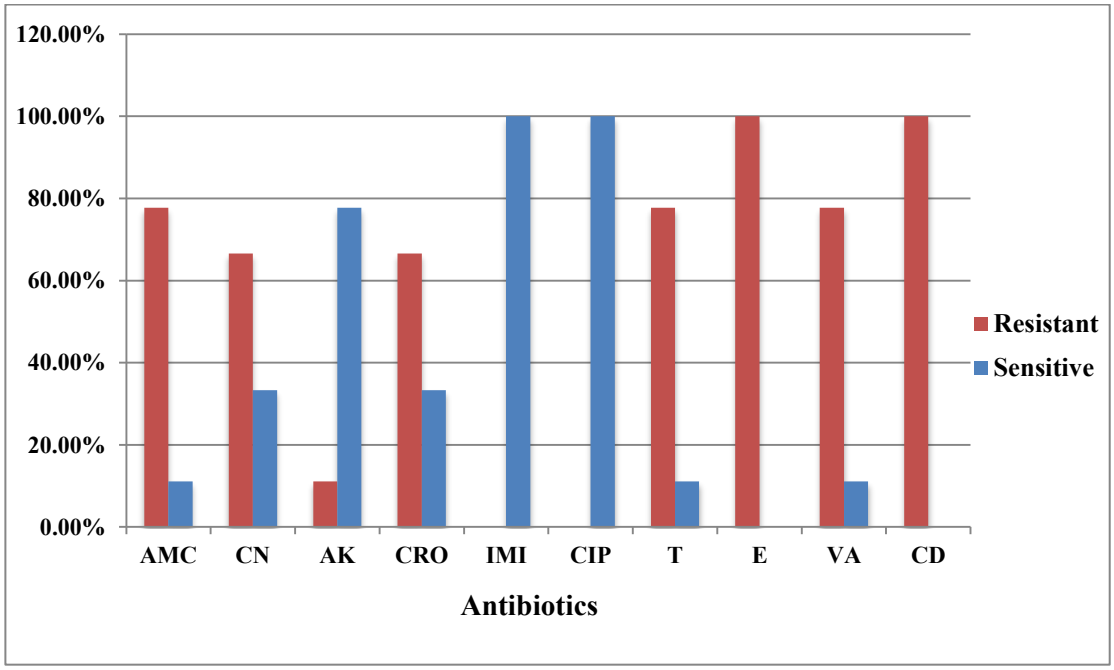

Figure 2- The results of antibiotic susceptibility test for Gram-Positive bacteria

Conclusion

In conclusion, honey can be used as antibacterial agent against bacterial species regardless of the bacteria are belong to the gram positive or gram negative group. The great value of antibacterial effect can be obtained when honey at concentration of 100% and the inhibition effect decreased as honey diluted. The antibacterial activity can be influenced by the honey sample, included the floral source and the bee colony nature. Additionally the antibacterial activity of honey depended on the type of bacterial species, which means the antibacterial effect can be changed from strain to another.

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