

Original Research Article

Antibacterial Activity of Bee and Yemeni Sidr Honey Against Some Pathogenic Bacterial Species

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A B S T R A C T

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Honey has the ability to fight food-borne pathogens as *E. coli* and *salmonella*, and other certain bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial activity of local Isis and Yemeni Sidr honeys against *Salmonella typhi*, *Neisseria meningitides*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Shigella flexneri* and *Proteus vulgaris* were evaluated. Disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, growth curve patterns were used in this investigation. The findings indicated that both honey samples had growth inhibitory effect on all tested bacteria. Increasing the honey concentration increased the inhibition of growth of the tested bacteria. Yemeni Sidr honey was more potent than Isis honey in producing the inhibitory growth effect as an antibacterial agent. Isis and Sidr Yemeni honeys different dilutions were more effective against *E.coli* than other bacteria. MIC of Yemeni Sidr honey samples ranged from 10 to 20 mg/mL for the tested organisms while the MBC of Yemeni Sidr honey samples ranged from 20 to 80 mg/mL. We are of the opinion that Isis and Yemeni Sidr honeys could potentially be used as therapeutic agents against bacterial infection particularly to the tested microorganisms

Introduction

Honey has a long medicinal history. The ancient Egyptians made offerings of honey to their gods, used it as an embalming fluid and a dressing for wounds. On that last point, at least, they thought it do something. Today, many people swarm to honey for its antibacterial and anti-inflammatory properties. Holistic practitioners consider it one of nature's best all-around remedies (Paul, 2007). The fact that honey has antibacterial properties was recognized for

more than a century because it cures infections (Subrahmanyam et al., 2001). Honey resistance has never been reported nor any toxicity or side effects, low cost of maintenance, and local availability confer valuable advantages to using honey as an alternative antimicrobial therapy (Zainol et al., 2013) There are numerous reports of the antimicrobial activity of honey against a wide range of bacterial and fungal species (Chute, 2010; Kwakman et al., 2010). The

antimicrobial activity could be attributed to osmotic effect of honey, the low pH of honey being between 3.2 and 4.5 (Cooper et al., 2002), hydrogen peroxide, defensin-1, as well as the presence of phytochemical factors (Frankel et al., 1998).

Thereby, the inhibitory activity caused by the osmotic effect of honey dilutions obviously depends on the species of bacteria. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (Ahmed, 2012).

Several types of bacteria, commonly involved in wound infections like *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* spp., *Streptococcus faecalis* and *Pseudomonas aeruginosa*, are susceptible to the antibacterial activity of honey regardless to their resistance to antibiotics (Lusby et al., 2005; George and Cutting, 2007; Cooper, 2008).

In vitro studies support the antimicrobial effect of honey against a wide range of pathogens including β -haemolytic streptococci, methicillin-resistant *S. aureus* and *Pseudomonas sp.* (Cooper and Molan, 1999). In vivo studies are less conclusive but honey has been used to treat burns (Manisha and Shyamapada, 2011) and meningococcal lesions (Dunford et al., 2000; Manisha and Shyamapada, 2011). Subrahmanyam (1998) compared between honey and silver sulphadiazine on treatment of patients with burns and found less inflammation, lower infection rates and faster healing in patients treated with honey.

This study aimed to investigate the antibacterial activities of Yemeni Sidr honey

and local bee honey against some pathogenic microorganisms, also for comparing the growth curves of some tested gram positive and gram negative bacteria before and after exposing to Yemeni Sidr honey

Materials and Methods

Bacterial strains

The following control bacteria strains, standard test organism and clinical isolates most commonly involved in causing gastroenteritis, pneumonia, wound and urinary tract infection were used (ATCC, US) . Control [*Salmonella typhi* (ATCC: 14028), *Neisseria meningitides*(ATCC: 13090), *Shigella flexneri* (ATCC: 12022), *Escherichia coli*(ATCC: 25922),*Klebsiella pneumoniae* (ATCC:13883), *Staphylococcus aureus* (ATCC: 25923), *Pseudomonas aeruginosa*(ATCC: 27853),*Haemophilus influenza* (ATCC: 35056), And *Proteus vulgaris* (ATCC:13315)].

These were sub cultured on Nutrient agar (Lab M, UK) and incubated aerobically at 37°C. Organisms were maintained in the laboratory on nutrient agar slopes at 4°C (Cappuccino and Sherman, 1995).

Honey samples

Two honey samples were used in this study, one obtained from local market in Egypt (Isis Honey) and the other from Saudi Arabia (Yemeni Sidr Honey) and stored in the dark at room temperature.

Different concentrations of each honey constituting, 10, 20, 40, 60 and 80% (v/v) were made using sterile distilled water. This was done by dissolving the respective volumes: 1, 2, 4, 6, 8 ml of each honey into corresponding volumes of sterile distilled

water to give a 10 ml preparation (Alqurashi et al., 2013).

Antibacterial activity

The disc diffusion technique was employed as previously described by Bauer et al (1966). Discs impregnated with the different concentrations of each honey were employed in the study. 0.5 McFarland standard was prepared by the method of Koneman et al (1992) and the turbidity adjusted to 1.5×10^8 CFU/mL (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the nutrient agar plates.

The plates were allowed to dry for 3 to 5 min. Thereafter, all antibiotic discs were placed on the inoculated plates and were incubated for 24 h at 37°C. They were then examined and the diameter of the zone of inhibition was measured in mm. The experiment was repeated in triplicates for each isolate.

Minimal Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Serial dilutions of the two honey samples were made in test tubes that contained 1 ml of Mueller Hinton broth medium to give a final concentration of 80, 40, 20, 10, 5, 2.5, 1.25 and 0.62 mg/ml. 20 µl of the test organisms (1.5×10^8 CFU/ml) was dispensed into the tubes. Negative control tube just contained 1 ml of honey but no organisms. Positive control tubes contained only 1 ml broth medium and each of the organisms but no honey. The tubes were incubated at 37°C for 24 h. After incubation, turbidity of each tube was visually inspected. Clear test tube indicated break point (Mackie and McCartney, 1996). From the tubes showing

no visible sign of growth or turbidity in MIC determination, test microorganisms were inoculated onto sterile nutrient agar plates by streak plate method. The plates were then incubated at 37°C for 24 h. The least concentration that did not show growth of test organisms was considered as the MBC.

Determining the growth curves of bacterial cells exposed to the MBC of Sidr honey:

To examine the growth curves of bacterial cells exposed to the MBC of Sidr honey, four common gram positive and gram negative bacteria were chosen as two strains were highly affected by Sidr honey and 2 strains were partially affected (*S.aureus*, *N.meningitidis*, *E.coli* and *P.aeruginosa*). They were cultured on Mueller-Hinton broth and the bacterial cell concentration was adjusted to 0.5 McFarland standards. They inoculated with 20 µl of their specific MBC concentrations of Sidr honey.

Each culture was incubated in a shaking incubator at 37°C for 24 h. Growth curves of bacterial cell cultures were attained through repeated measures of the optical density (O.D.) at 600 nm. Then the Heterotrophic plate count (HPLC) were done for each O.D reading.

Result and Discussion

The inhibition zone diameter (IZD) of different Egypt Honey (Isis) and Yemeni Sidr honey concentrations (80-10%) were determined for *Salmonella typhi*, *Neisseria meningitides*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Shigella flexneri*, *Neisseria meningitidis* and *Proteus vulgars*. Both Yemeni Sidr honey and Egyptian honey were highly effective against

Salmonella typhi, *Neisseria meningitides*, *Shigella flexneri*, *Escherichia coli*. The effect on *Klebsiella pneumonia* and *Staphylococcus aureus* were less than other bacteria showing Yemeni Sidr honey more effective than Egyptian honey while there were no effect of both types of honey on *Pseudomonas aeruginosa*, *Haemophilus influenza* and *Proteus vulgaris*. (Fig1 and 2).

As Yemeni Sidr honey was the highly effective the MIC and MBC against the tested organisms were determined (Fig 3). The MIC was 20 mg/ml for *Neisseria meningitides*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginos* while it was 10 mg/ml for *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Haemophilus influenza* and 40% for *Proteus vulgaris*. The MBC was found to be 40 mg/ml for *Salmonella typhi*, *Neisseria meningitides*, *Shigella Flexner*, *Pseudomonas aeruginosa* and *Haemophilus influenza*, while it was 20 mg/ml for *E.coli* and 60 mg/ml for *Klebsiella pneumoniae* and *Staphylococcus aureus* but it was 80 mg/ml for *Proteus vulgaris*

Figure (4) showed that after the first 2 hours *E. coli* growth increased until the 9th hour then it began the stationary phase while a re-growth was shown at the hour 15 then decreased again, while after treatment with Yemeni Sidr honey there were nearly no growth at the first 15 hours but there was a slight re-growth at the hour 15 then it decreased again.

In figure (5) it was obvious that after the first 4 hours the bacterial growth increased until the 8th hour then it began the stability phase while a re-growth was shown after the hour 13 to the hour 17, while after treatment the growth were inhibited completely as there was no growth at the treated 17 hours.

The figure showed increased growth of *S.aureus* after the third hour to the fifth hour then became stable to the seventeenth hour, but after treatment the growth were inhibited completely to the third hour then increased slightly to the 16th hour and decreased again at the 17th hour.

Figure (7), illustrated increased growth of *Neisseris meningitidis* from the first to the fifteenth hour then became nearly stable at hour 16 and markedly decreased at the 17th hour, but after treatment there were no growth all over the 17 hours.

Figure(8) represented the heterotrophic plate counts for *E. coli* , *P. aeruginosa*, *S. aureus* and *N. meningitidis* it showed that the highest colony count number before treatment was for *Pseudomonas aeruginosa* compared to the other three tested strains while all the treated types of bacteria showed the least colony count number revealing the powerful effect of Yemeni Sidr honey.

In our study, two honey samples were tested for their antimicrobial activity on *Salmonella typhi*, *Neisseria meningitides*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* ,*Haemophilus influenza* , *Shigella flexneri* and *Proteus vulgaris*. The present study showed varying degree of *in vitro* growth inhibition activity of Isis and Yemeni Sidr honeys against the tested organisms. These might be due to the osmotic effect, the effect of pH, and the sensitivity of these organisms to hydrogen peroxide which are unsuitable for bacterial growth, represented as an inhibition factor in honey (Postmes et al., 1993; Minisha and Shyamapada, 2011). Our result was supported by a number of previous studies which have demonstrated that various honeys, both commercially and locally

produced, have antibacterial activity. Nzeako and Hamdi (2000) in their study of six commercial honeys found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur at honey concentrations 40%. In contrast to the current study, these authors also found that honey inhibited *C. albicans*, although the zone of inhibition was small compared with other organisms. Ceyhan and Ugar, 2001 tested 84 honeys against eight bacteria and two fungi showing that honey has broadspectrum activity. In addition, these authors found that the antibacterial activity of honey was greater than that which could be attributed to the sugar content of the honey. The antibacterial activity of honey has also been investigated for its potential use in reducing food-borne pathogens (Taormina et al., 2001), preventing catheter exit/entry site infection (Quadri and Huraib, 1999), for the treatment of colitis (Bilsel et al., 2002) or even to protect the gastric mucousin *H. pylori*-induced inflammation (Osataet, 1999 and Ali, 2003). The application of honey to wounds to animals in veterinary environments has also been noted (Mathews and Binnington, 2002).

All the different concentrations of both honey samples (10 to 80%) showed growth inhibitory activity against *E. coli* more than other bacteria tested. This was in contrasts with the result reported by (Hegazi, 2011; Hegazi and Fyrouz, 2012) who reported that the different types of Saudi honey were less inhibitory against *E. coli* than other bacteria. All the tested bacteria were sensitive to Isis and Yemeni Sidr honeys at 40 to 80% concentrations. The antibacterial activity of Yemeni Sidr honey was higher than those obtained by Isis honey. Variations seen in overall antibacterial activity were due to changes in the level of hydrogen peroxide

achieved and in some cases to the level of non peroxide factors. The content of non peroxide factors was obviously related to the floral source and sometimes accounted for the major part of the antibacterial activity in honey (Alqurashi et al., 2013). Molan and Cooper (2000) reported that the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source. This result was in agreement with those previously reported by Mohammed et al (2008). The different concentrations of the two honey samples had good growth inhibitory effect on the tested microorganisms. Similar result was previously reported by Mohapatra et al (2011) for *E. coli* and *P. aeruginosa*, (Agbaje et al., 2006) for *E. coli*, *K. pneumoniae* and (Hern et al., 2009) for *Haemophilus influenza*. The less inhibition effect of the two tested honey against *K. pneumoniae* and *S. aureus* was in agreement with Patricia et al (2005) who reported that the overall poor activity of the honeys against *S. aureus* was unexpected as previous reports have shown that Maunka honey has an excellent activity against this organism. For example, Cooper et al (1999), who also used an agar dilution method, demonstrated that the minimum inhibitory concentration for Maunka honey against 58 strains of *Staphylococcus* was 2–3% (v/v) and for pasture honey 3–4% (v/v).

Part of the explanation for the difference in results of Patricia et al (2005) may be due to methodological differences between studies because the agar dilution method used by Cooper et al (1999) was slightly different from that used in his study. However, it was also likely to be due to variation in the composition of the honey being used by visual inspection.

Figure.1 Inhibitory growth activity of Yemeni Sidr honey against *Salmonella typhi*, *Neisseria meningitides*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Shigella flexneri* and *Proteus vulgaris* using disc diffusion test

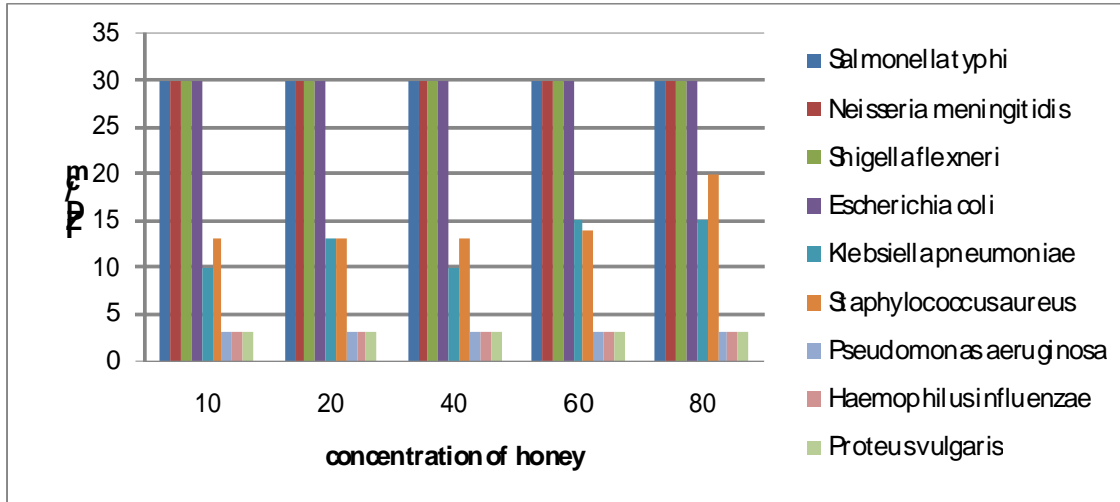
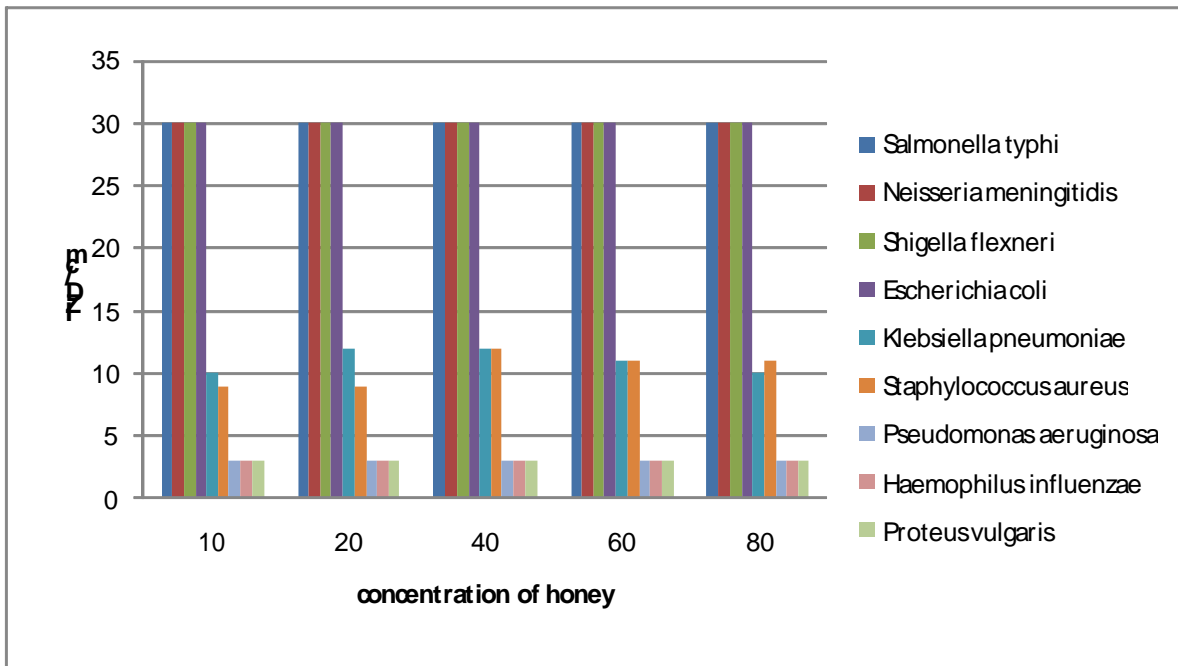


Figure.2 Inhibitory growth activity of ISIS honey against *Salmonella typhi*, *Neisseria meningitidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Shigella flexneri* and *Proteus vulgaris* using disc diffusion test



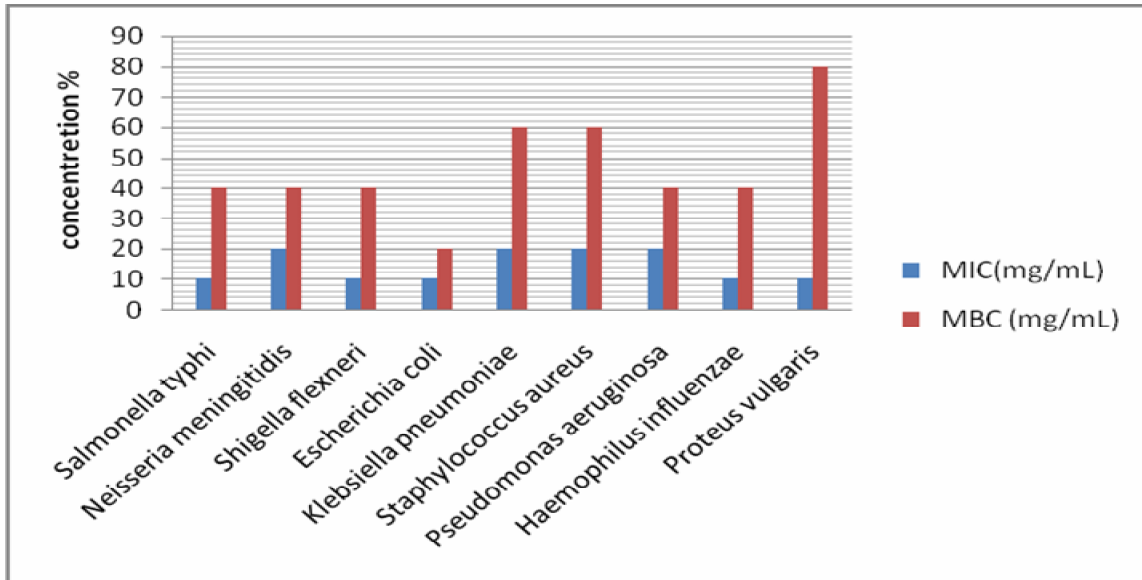


Figure.3 The (MIC) and (MBC) of Yemeni Sidr honey against different tested organism

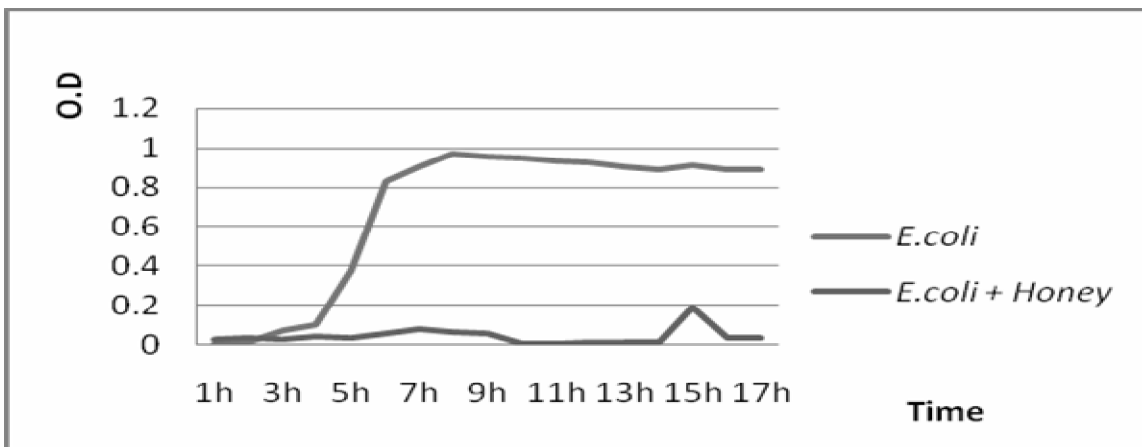


Fig.4 Growth pattern of *E.coli* before and after treatment with 20% Sidr honey (MBC)

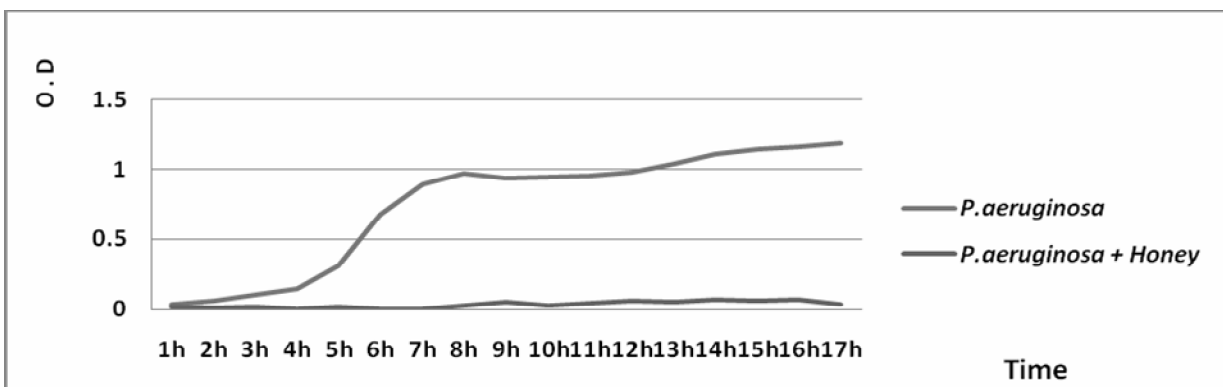


Fig.5 Growth pattern of *P.aeruginosa* before and after treatment with 40% Sidr honey (MBC)

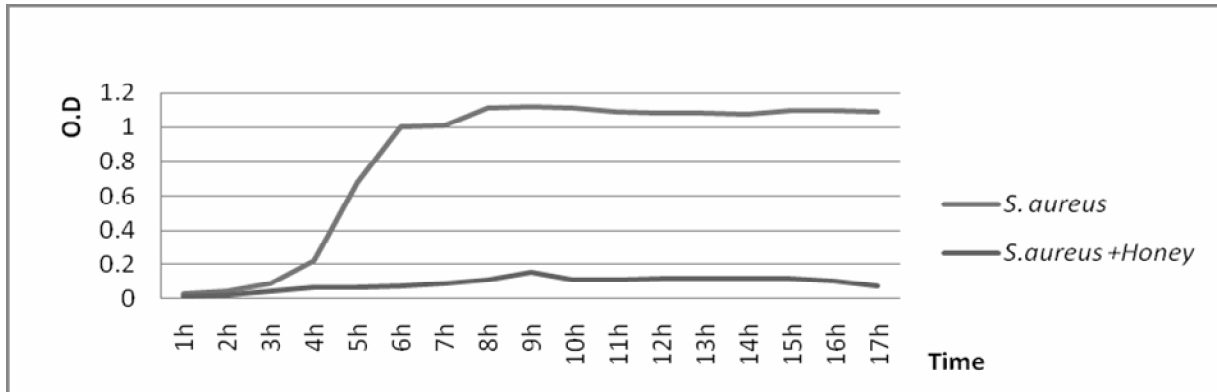


Fig.6 Growth pattern of *S.aureus* before and after treatment with 60% Sidr honey (MBC)

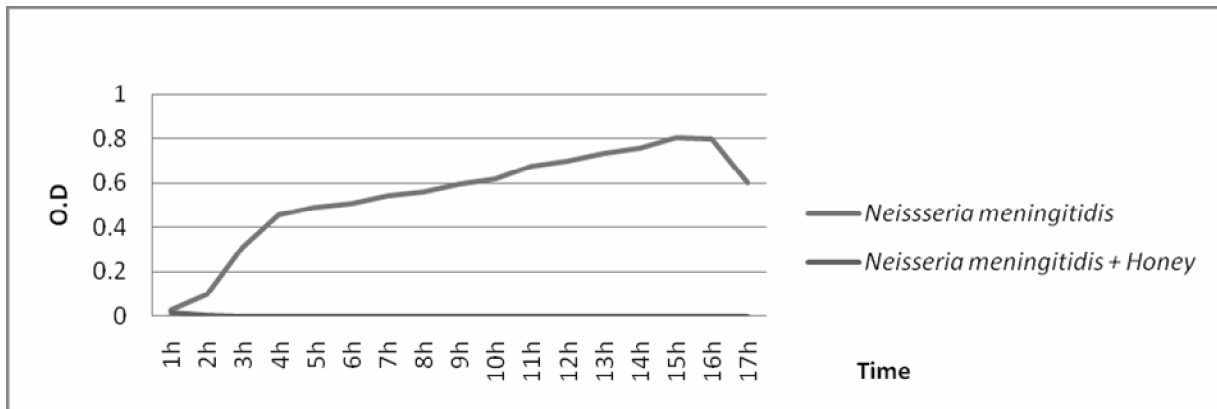


Fig.7 Growth pattern of *Neisseria meningitidis* before and after treatment with 40% Sidr honey (MBC)

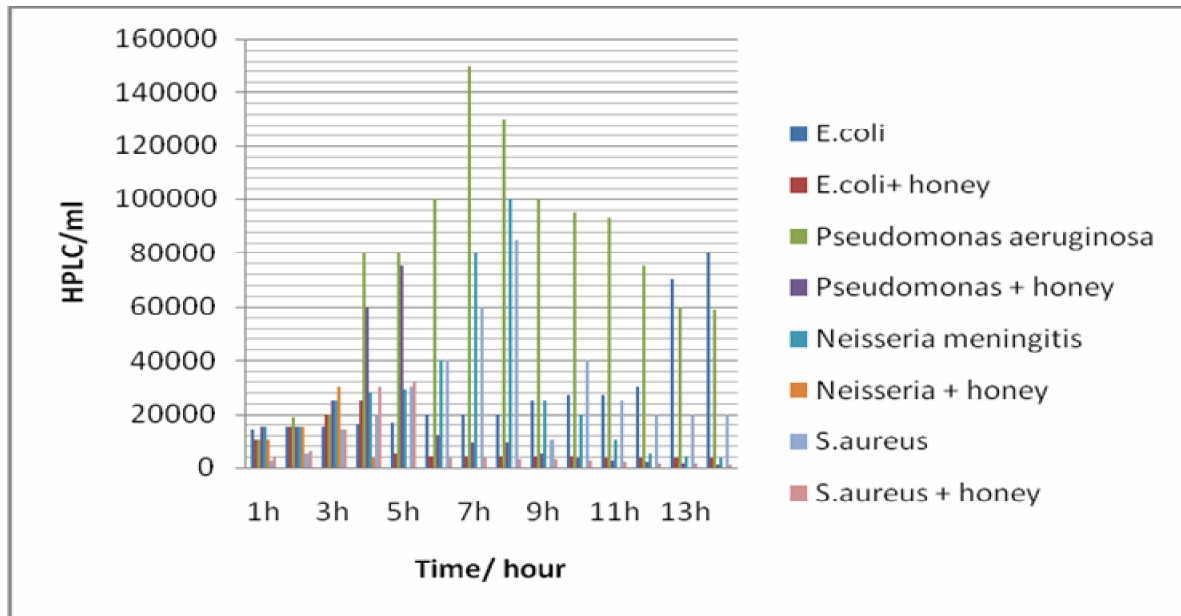


Fig.8 Heterotrophic plate count (HPLC/ml) of *E.coli*, *Pseudomonas aeruginosa*, *Neisseria meningitidis* and *S.aureus* before and after treatment with Yemeni Sidr honey

The present findings are supported by Patricia et al (2005) and Kwakman et al (2008) who showed that all honeys tested have some antibacterial action from concentrations as low as 5%; however, the greatest inhibition is seen at 20%. The MBC value of Yemeni honey sample was in the range of 20 to 40 mg/ml. The lowest MBC value (20 mg /ml) was against *Escherichia coli*. The present findings were in agreement with (Hern et al., 2009). Comparing the mean \pm standard deviation of the inhibition diameters of the tested bacteria at different honey concentrations, we observed that there was statistically significant difference in the values ($P \leq 0.05$) between microorganisms at all the honey concentrations. Our results further show that there was an increase of inhibition zone for the tested microorganisms with increase in the concentration of honey. This was obvious by statistical analysis which revealed that there was significant difference in the values ($P \leq 0.05$) between the different honey concentrations.

Our study of bacterial growth pattern and Heterotrophic plate count of *E.coli*, *Pseudomonas aeruginosa*, *Neisseria meningitides* and *S.aureus* before and after treatment with Yemeni Sidr honey showed that the highly effect of Sidr honey was on *Neisseria meningitides* then *P.aeruginosa* then *E.coli*. Similarly, Wilkinson and Cavanagh, 2005 compared the activity of 13 honeys at four concentrations (10, 5, 2.5, and 1% v/v) with corresponding dilutions of an artificial honey, a solution containing the principal sugars found in honey and using *E. coli* and *P.aeruginosa* as the test organisms. In vitro antimicrobial activity of honey was reported by Coates (2002) and Mohapatra et al (2011) who observed that honey stopped the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). Honey has a potent antibacterial activity and

is very effective in protecting wounds from infection (Mohapatra, 2011).

In light of the enormous potential for application of honey within a clinical environment, it is important that research continues not only into those honeys recognized as antibacterial, but also into other locally produced, as yet untested, honeys.

The present study revealed that Isis and Yemeni Sidr honeys were effective in inhibiting the *in vitro* growth of *Salmonella typhi*, *Neisseria meningitides*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Shigella flexneri* and *Proteus vulgaris*.

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