ORIGINAL ARTICLE

Antibacterial Activity of Honey Against Bacteria Isolated from Respiratory Tract Infections

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ABSTRACT

Objective: This study aims to verify any antibacterial activity of honey against bacteria concerned with Respiratory Tract Infections in Karachi, Pakistan.
Methodology: Three brands of commercial honey, one 'crude' and two 'processed' were used to determine their antibacterial activity against four bacterial species, *Klebsiella pneumoniae* (n=56), *Pneumococci* (n=34), *Pseudomonas aeruginosa* (n=16), and *Staphylococcus aureus* (n=20), isolated from Sputum and Throat swab specimens. The 'cork-bore method' was employed using Mueller-Hinton agar , and the inhibition zones around the wells containing 20%w/v honey solutions were assessed using Imipenem disc (30ug) as the standard of antibacterial activity. Data was analyzed using SPSS 16.0.
Results: Significant antibacterial activity of honey was observed against the 4 RTI isolates, with higher sensitivities for ProcS (Salman's Honey) and Crude sample. The Crude sample yielded relatively superior inhibition zones than the other two specimens; it affected all 34 (100%) of *Pneumococci*, 29 of 56 (52%) *Klebsiellae*, 14 of 20 (70%) *Staphylococci* and 4 of 16 (25%) *Pseudomonas* isolates. *Pneumococci* were significantly more sensitive to all 3 honey specimens, while *pseudomonas* were least sensitive compared to other isolates.
Conclusion: Samples of honey showed promising *in vitro* antibacterial activity on some RTI isolates.

Key words: Honey, antibacterial sensitivity, respiratory infections.

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OBJECTIVE

"To verify the antibacterial activity of honey against bacteria responsible for causing Respiratory Tract Infections (i.e., *Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus and Klebsiella pneumoniae*)."

INTRODUCTION

With antibiotic resistance on the rise[1] and no new group of antibiotics being added for quite a long time, the need for a new antibiotic becomes imperative. Synthetic antibiotics have established side effects on systemic administration; such as hypersensitivity reactions, nephrotoxicity, hepatotoxicity and GI upset etc. Therefore we need a natural antibiotic with least adverse effects on human body.

In this regard we get a clue from Holy Quran and Hadith, where "honey" has been declared a substance of healing:

"And your Lord inspired the bees, saying:" Take your habitations in the mountains and in the trees and in what they erect. Then, eat of all fruits, and follow the ways of your Lord made easy (for you)." There comes forth from their bellies, a drink of varying colour wherein is healing for men. Verily, in this is indeed a sign for people who think." al-Nahl (the Honey Bee): *16:68–69*

Prophet Muhammad (s.a.w.) said,

"Make use of the two cures: honey and the Qur'an." [Ibn Majah]

"*If there is any good in your medicines, then it is in a gulp of honey, a cupping operation (arabic hijamah), or branding (cauterization), but I do not like to be (cauterized) branded."(Sahih Bukhari 603)*

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Having been used for its healing properties since ancient times, today studies reveal its effectiveness in a number of medical conditions like Wound-healing,[2] Diabetic ulcers,[3] Diarrhea,[4] Colitis,[5] Seborrhea and dandruff,[6] Gastro-intestinal ulcers[7] etc.

Globally, Respiratory Tract Infections (RTI) cause over 50 million deaths and are the most often reason for physician visits and prescription of antibiotics. In Pakistan, Acute Respiratory Infection is the number one (51%) most widespread and lethal disease, especially in children.[8,9] This high incidence is mainly due to malnutrition which leads to poor immunity.[10] We propose honey to be introduced as an antibiotic in RTI. It can not only serve as a cheaper antibiotic but also a complete nutraceutical. It comprises of carbohydrates (mainly fructose and glucose), Water, Organic acids, Amino acids, Minerals (calcium, sodium, phosphorus, magnesium, silicon, iron, manganese, and copper.), Enzymes (invertase, amylase), vitamins (A, B Complex, C, D, E) and antioxidants (Pinocembrin, Ascorbic acid, catalase and selenium)[11]

In this research, we take this opportunity to work on honey, which is described as a panacea in Islamic teachings, and explore its efficacy as an antibacterial in 'Respiratory Tract Infections' through scientific methods.

MATERIALS AND METHOD

Bacterial Strains: Four bacterial species, *Klebsiella pneumoniae* (n=56), *Streptococcus pneumoniae* (n=34), *Pseudomonas aeruginosa* (n=16), and *Staphylococcus aureus* (n=20) were isolated from Sputum and Throat swab specimens of patients presenting with RTI at a local diagnostic lab in Karachi.

Preparation of test samples: Samples of processed honey Salman's (ProcS), processed honey Langnese (ProcL) and Crude honey were procured from the market in order to determine their antibacterial properties. 10mg of honey samples were taken in 50ml sterile volumetric flask. The volume was made up with sterile distilled water for a dilution of 20%w/v.[12]

Antibacterial assay: The tests were run in Mueller Hinton agar (Oxoid UK) *for Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumonia*; and Chocolate agar for S*treptococcus pneumonia*e. Wells of 5ml diameter were cut with sterile cork borer in the inoculated agar taking special precaution of sterilizing the borer with ethanol every time. The wells were filled with test samples with the help of sterile droppers. Similarly in control plates, wells were filled with sterile distilled water. The plates were incubated 24 hours at 37°C. At the end of incubation period, the inhibition zones were observed and noted down for 'Sensitive', 'Intermediate' or 'Resistant' using Imipinem Disc (30µg) as the standard of antibacterial activity.

Data Analysis: This was done by applying non-parametric Test Kruskal-Wallis Test, one- way ANOVA and Post Hoc Test for multiple responses using SPSS 16.0.

RESULTS

All 3 honey samples show significant antibacterial activity ($p<0.05$) against *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pneumoniae (Pneumococci)* (Table I). Comparatively samples ProcS and Crude had higher activity as compared to ProcL; while Crude being more active than ProcS. Pneumococci show highest susceptibility for honey while pseudomonades are relatively less susceptible.

Figure I: Percentage antibacterial activity of antibacterial activity of crude honey

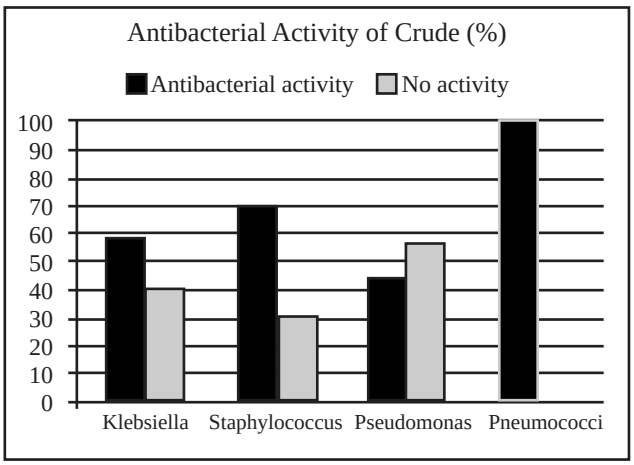

Figure II: Percentage antibacterial activity of antibacterial activity of procs (salman's honey)

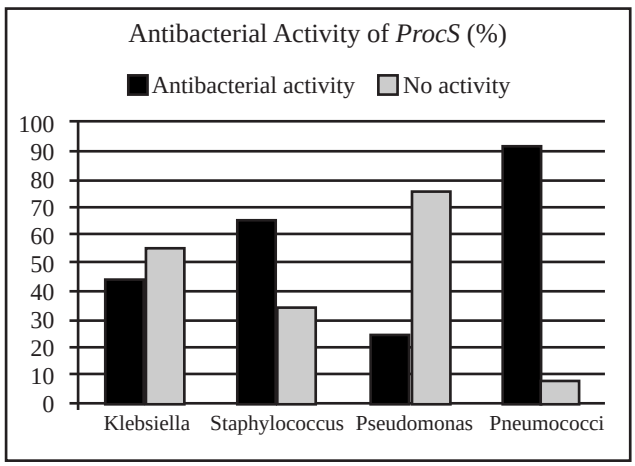

Table I: Zones of inhibition produced by honey samples against rti bacteria

Honey Samples	Name of organism	Sensitive (n)	Intermediate (n)	Resistant (n)	Total (n)	% Antibacterial activity*
Crude	*Klebsiella pneumoniae*	29	4	23	56	58.92%
	Staphylococcus aureus	14	-	6	20	70%
	Pseudomonas aeruginosa	4	3	9	16	43.75%
	Streptococcus pneumoniae	34	-	-	34	100%
ProcS	*Klebsiella pneumoniae*	20	5	31	56	44.64%
	Staphylococcus aureus	11	2	7	20	65%
	Pseudomonas aeruginosa	1	3	12	16	25%
	Streptococcus pneumoniae	30	1	3	34	91.17%
ProcL	*Klebsiella pneumoniae*	19	3	34	56	39.28%
	Staphylococcus aureus	10	2	8	20	60%
	Pseudomonas aeruginosa	3	2	11	16	31.25%
	Streptococcus pneumoniae	31	2	1	34	97.05%

Key: Crude = Crude sample, *ProcS* = Salman's honey, ProcL= Langnese honey, *Antibacterial activity= Sensitive + Intermediate

Figure III: Percentage antibacterial activity of antibacterial activity of procl (langnese honey)

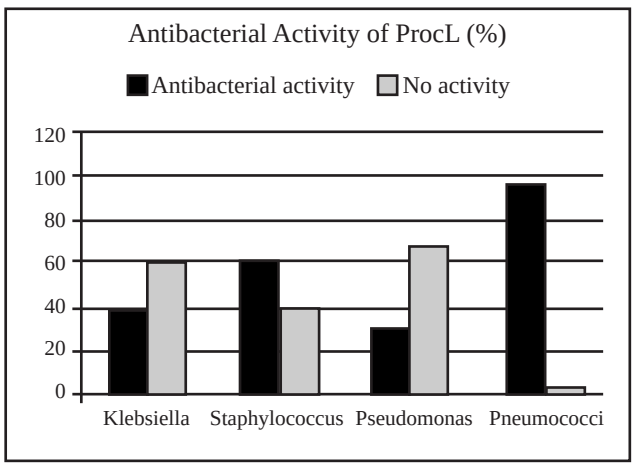

Figure IV: Comparison of antibacterial activities of 3 Honey samples

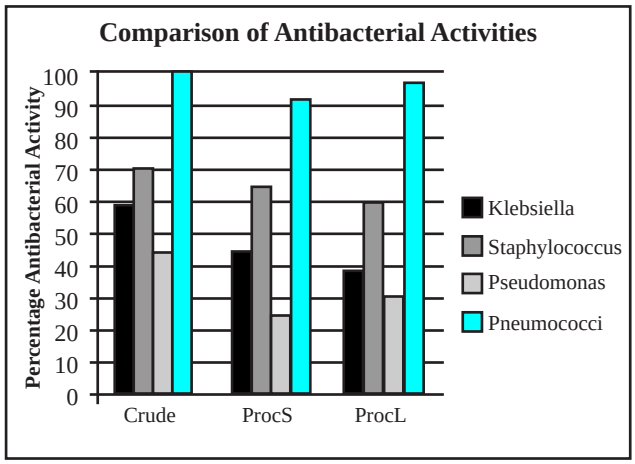

Figure V: Inhibition zones of pseudomonas

DISCUSSION

Work on antibacterial activity of honey was begun in late 20th century. With the increasing trend of resistance to antibiotics, researchers developed interest towards the properties of honey.

Majority of national and international researches have demonstrated honey's antibacterial activity against pathogens of gastrointestinal and urinary tract infections like Salmonella,[12] E Coli.[13] This study focuses on the clinical isolates of RTI only. We selected respiratory tract because it has a direct influence of the changing environment like industrialization and air pollution. When honey is taken, it is acts topically on upper respiratory tract and after being absorbed in the blood, it acts on lower respiratory tract as well. So far, only one study from Canada focused on honey s antibiotic activity in Chronic Sinusitis.[14]

Staph aureus causes pneumonia secondary to viral infections which is associated with high rate of complications; Pseudomonas causes mostly hospital –acquired infections while Klebsiella more commonly affects debilitated and malnourished individuals[26] Among the four species that we took, Staph aureus and Pseudomonas have been most worked on. In Pakistan, studies have shown significant activity of

honey against Staph aureus, Pseudomonas and Klebsiella.[12,17] In an Indian study, 20% dilution shows no zones of inhibition for Staph aureus and for higher concentrations for Klebsiella as well,[15] whereas in our study significant antibacterial activity is shown against these species at the same concentration (Table I). However, study from Japan demonstrated non-peroxide antibacterial activity of honey against Staph aureus and Pseudomonas[16] and one from Canada showed antibacterial activity against Staph aureus.[18] While another study from Canada shows 100 percent activity of honey against planktonic forms of Staph Aureus and Pseudomonas and significantly higher activity than the commonly used antibiotics against biofilms-grown isolates.[14]

Therefore our results are in conformity with positive and sifnificant findings from prior researches for *Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa* .

Our study is the first one to work on *Streptococcus Pneumoniae* (Pneumococci) which are a frequent cause of Pharyngitis and Pneumonia. In Pakistan, Pneumococci are becoming less susceptible to antibiotics day by day.[19] In our study, it was found to be the most susceptible bacterial species for honey, showing 100% susceptibility to one of the honey samples (Figure IV).

The Antimicrobial properties of honey can be attributed to several factors like high osmotic pressure, low water activity and pH.[20,21] However, in vivo, when these factors may be nullified due to dilution by body fluids, its Glucose Oxidase enzyme releases hydrogen peroxide when honey is diluted, oxygen is available and the acidity of honey is neutralized by body fluids. On dilution of honey this enzyme activity increases by a factor of 2,500 - 50,000, thus giving a "slow-release" antiseptic at a level which is antibacterial but not tissue-damaging. In situations where H2O2 is scavenged by body's catalase, the non peroxide factors play their role. These include Methylglyoxal (MGO) and Phytochemical factors, which are complex phenols and organic acids often referred to as "flavonoids" (e.g., phenolic acids, caffeic acids). These complex chemicals are unaffected by dilution, heat or light. Presence of these antibacterial factors is confirmed by treating honey with catalase to remove the hydrogen peroxide activity.[22]

Along with its antibacterial activity, Honey can give symptomatic relief in RTI as well. Honey's antioxidant constituents and its ability to chelate free iron give it an anti-inflammatory activity.[23] It boosts up immune system by promoting Lymphocytes proliferation and supporting monophages.[11] It has been shown to have effectiveness in cough,[24] in fact better than cough medicines.[25]

We recommend more research to be done on honey, especially on pharmaceutical grounds, to establish its role as an effective antimicrobial.

CONCLUSION

This study is a humble effort to scientifically elaborate the signs of Holy Quran and the Ahadith. It shows that Honey has promising antibacterial activity against RTI bacteria with a relatively higher sensitivity against Pneumococci. Respiratory tract infections are commonly encountered and in this era of ever-increasing antibiotic resistance, use of this non toxic and inexpensive natural antibacterial should be encouraged and further researched upon.

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