

Antimicrobial Activity of Honey on Pathogens Isolated from Patients with Allergic Respiratory Diseases

Kishori Tarfe^{1*} and Sunita Deshpande²

¹Department of Biotechnology, Smt. Chandibai Himathmal Mansukhani College,
Ulhasnagar, Maharashtra, India

²Former Professor, Seth G. S. Medical College & KEM Hospital, Parel, Mumbai, Maharashtra, India

*Corresponding Author E-mail: kishorikambli@gmail.com

Received: 27.05.2018 | Revised: 25.06.2018 | Accepted: 29.06.2018

ABSTRACT

Conventional medical treatments for allergic respiratory diseases include corticosteroids sometimes combined with antibiotics. Prolonged treatment with corticosteroids & antibiotics may lead to suppression of immune response as well as emergence of drug resistant strains. Therefore there is always need for alternative treatment. Honey has been used in medicine since ancient time. Both these traditional natural products have a wide range of activity against variety of pathogens. 140 sputum samples collected from patients with allergic respiratory diseases such as Asthma & ABPA, were tested for presence of pathogens. Total 20 bacterial isolates (*K. pneumoniae*, *Staphylococcus aureus* & *Pseudomonas aeruginosa*) & 12 fungal isolates (*Candida species*, *A. fumigatus*) were obtained. These isolates were tested for their susceptibility towards two varieties of Honey, Karvi & Jambul by Agar cup & Agar dilution method. Jambul Honey was found to be more effective as compared with Karvi Honey. Honey varieties tested were found to be most effective at concentration 50% against *Staphylococcus aureus* followed by *Klebsiella pneumoniae*, *P. aeruginosa*, *Candida species* & *A. fumigatus*. Although Honey has been given as home remedy, both have remarkable activity on pathogens isolated from allergic respiratory infections. Presence of pathogens in respiratory tract of patients with respiratory allergies increases severity of their symptoms; therefore along with other medications Honey should be included in their treatment. Both Jambul & Karvi Honey are Indian honeys with great antimicrobial potential.

Key words: Asthma, ABPA, Karvi Honey, Jambul Honey.

INTRODUCTION

As bacterial infections play a major role in etiopathogenesis of allergic respiratory infections their consequent treatment is required by means of wide spectrum antibiotics as well as prescription of bacterial immunotherapy^{1,2}. Bacteria commonly found

to be associated with respiratory allergies include *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *H. influenzae* etc^{3,4}. Along with these organisms *Candida species* is also found as important pathogen in patients with respiratory allergies.⁵

Cite this article: Tarfe, K. and Deshpande, S., Antimicrobial Activity of Honey on Pathogens Isolated from Patients with Allergic Respiratory Diseases, *Int. J. Pure App. Biosci.* 6(3): 388-397 (2018). doi: <http://dx.doi.org/10.18782/2320-7051.6564>

Bacterial lipopolysaccharide plays a major role in asthma. Inhaled lipopolysaccharide can exacerbate airway inflammation and airway obstruction in allergic asthmatics. As bacterial infections play a major role in etiopathogenesis of allergic respiratory infections their consequent treatment is required by means of wide spectrum antibiotics as well as prescription of bacterial immunotherapy^{1,2}.

Bacterial antigens potentiate the action of inhalant allergens. The structural elements of bacteria and toxins produced by them intensify the release of mediators (Leucotrienes, histamine, IL1, IL 4, IL 6, IL 8 and TNF – alpha) of the inflammatory reactions^{6,5}.

Respiratory bacterial infections can also directly modulate T helper 1 and 2 selection parallel to the immune response to inhalant allergens. It has been also proved that in respiratory infection, bacteria hold the main responsibility in the inflammatory and bronchospastic response in the etiopathogenesis of asthma⁵.

Of all microbial products, endotoxin has been studied extensively. Along with endotoxins a variety of other microbial agents are known to have immune stimulatory properties which includes beta (1, 3) – glucans, bacterial DNA and other bacterial components. Beta (1, 3) glucans are glucose polymers present in the cell wall of most fungi and yeasts, some bacteria and vegetable materials⁷.

Inhaled LPS can exacerbate airway inflammation and airflow obstruction in allergic asthmatics. Allergic subjects are more sensitive than nonallergic subjects to the bronchoconstrictive properties of inhaled LPS. In addition prior allergen exposure significantly augments the inflammatory response to inhaled LPS⁸.

Fungi are known to causative factors that include asthma symptoms. Outdoor airborne fungi including *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* and indoor fungi like *Neurospora*, *Aspergillus* and *Euotinum* are significant triggers of IgE

formation. There is strong fungal/yeast component in the lung and/or gut microflora in individual with asthma. *Candida albicans* may be a prominent allergen for many people with asthma. The cell wall constituent mannan and acid protease an enzyme produced by *C. albicans* are both highly allergic and serum IgE antibodies are often increased in atopic individuals⁹. Allergic Bronchopulmonary Aspergillosis (ABPA) is also an important allergic disorder which is predominantly a disease of allergic subjects. ABPA is caused by hypersensitivity to *Aspergillus* antigen. *Aspergillus* species are ubiquitous occur worldwide and known to cause four distinct clinically and recognizable forms of hypersensitivity respiratory disorders i.e. Allergic Bronchopulmonary Aspergillosis (ABPA), Allergic *Aspergillus* sinusitis, IgE mediated asthma and hypersensitivity pneumonitis¹⁰.

Microbial infections associated with allergic respiratory infections increase the severity and duration of the disease as well as may themselves act as an allergen. Therefore their treatment with appropriate antimicrobials is essential. Many studies have suggested that patients with such associated respiratory infections when treated with antibiotics can improve patient's ability to breath. Antibiotic treatment helps in fast recovery of patient, which can reduce corticosteroid consumption by patients^{1,3}.

Long term use of steroids can give a lot of side effects including suppression of immune system making individual more prone to fungal infections similarly prolonged and irrational use of antibiotic may result in emergence of drug resistance organisms. Unnecessary and random use of medicines without prior diagnosis and also increases total cost of therapy.

An alternative for these antibiotics is use of natural products. They may act directly on the organisms by their inherent capacity of antimicrobial activity or may help in modifying the immune system which helps in control of symptoms of allergic respiratory infections.

From ancient times natural products like Honey is known to be effective against microorganisms and widely used as home remedy¹¹.

Honey has the potential to use in the treatment of skin infections, gastrointestinal conditions and eye infections¹². Honey is well known remedy for colds and mouth, throat or bronchial irritations and infections. It reduces and cures eye cataracts. Cures conjunctivitis and various afflictions of the cornea^{12,13}.

The Ayurvedic medicine uses Honey predominantly as a vehicle for faster absorption of various drugs such as herbal extracts¹². Honey may be used alone or in combination with other substances and has been administered both orally and topically.

It is well established that honey inhibits a broad spectrum of bacterial species. More recently honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram positive and gram negative bacteria. An antifungal action has also been observed for some yeasts, species of *Aspergillus* and *Penicillium*¹³.

The potential of Honey of subsiding respiratory symptoms can be used in treating exacerbations in allergic conditions. Such natural products are used regularly in Ayurvedic treatment but exact mechanism of action is not known as very little studies are documented in literature.

Although Honey is commonly used as home remedy to control severe exacerbations, it can be also used to control pathogens.

Objective of the study was to isolate bacterial and fungal pathogens from patients with allergic respiratory infections such as Asthma & ABPA. Further to evaluate the *in vitro* antimicrobial activity of natural products such as Honey (two varieties Karvi & Jambhul) & on isolates obtained from patients with respiratory allergies

MATERIAL AND METHODS

A total of 150 patients were included in the study.

These patients were enrolled for treatment of allergic respiratory disease such

as Asthma & ABPA & were visiting Medicine Department of T. N. Medical College & B. Y. L. Nair charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India.

These patients were located in different areas in Mumbai as well as outside Mumbai.

The senior clinician of Medicine department, T. N. Medical College did the selection of subjects on the basis of their clinical and radiological findings.

Patients selected were either hospitalized (Indoor basis) or outdoor patients at B. Y. L. Nair charitable Hospital.

Inclusion Criteria

1. Patients able to give productive sputum & clinically suspicious of infective etiology.
2. Patients who are able to produce brief clinical history.
3. Patients in whom antibiotics have not been administered within last 48-72 hours.

Following Exclusion Criteria were used while Selecting Patients for the Study

1. Patients who are not able to produce adequate sputum or give any other relevant respiratory samples.
2. Patients on prolonged antibiotic treatment.
3. Patients requiring ICU care.
4. Pregnant females.

Collection of Respiratory Specimens^{14,15}

Sputum samples were collected & processed by standard methods. Minimum 3 consecutive samples were studied for confirmation of results.

All sputum samples were graded by Murray & Washington grading system¹⁶ & unsatisfactory samples were discarded. Fresh samples were procured.

Processing of Respiratory Specimens^{14,15,17}

All specimens were studied microscopically as well as macroscopically. In macroscopic study characters like color, appearance, presence or absence of blood in specimens were noted.

Culture^{14, 18} – A loopful of each specimen (Representative portion) was inoculated or streaked on various culture media for isolation of pathogens. All aseptic precautions were taken while processing of samples.

Following media were used

1. Blood agar
2. Chocolate agar
3. MacConkeys agar
4. Sabouraud's Dextrose agar with tetracycline and chloramphenicol
5. Sabouraud's Dextrose agar with tetracycline, chloramphenicol and cyclohexamide

Study of following organisms was excluded

Anaerobic organisms, Mycobacterium spp., Protozoa & Viruses.

Above plates were incubated under appropriate conditions & for required time duration. After incubation colony characteristics of colonies obtained were studied.

Identification of the isolates obtained^{14, 19, 20}**Identification of *Klebsiella pneumoniae***

Colony characteristics & following biochemical tests

Indole test, Methyl Red test, VP test, Citrate test (IMViC), Motility, Lysine decarboxylase, production, Fermentation of Lactose, Sucrose, Sorbitol, Arabinose, Urease production

Identification of *Pseudomonas aeruginosa*

Oxidase test, Motility, Pigment production, Arginine decarboxylase production, citrate test, Nitrate reduction, Fermentation of Glucose.

Production of bright bluish green diffusible pigment was determined using nutrient agar. *P. aeruginosa* was distinguished from others by its ability to grow at 4°C.

Identification of *Staphylococcus aureus*

Colony characteristics & following biochemical tests

Coagulase test, Voges-Proskauer test, Nitrate reduction test, Urease test, Anaerobic fermentation of glucose & mannitol.

Identification of *Candida* species Colony characteristics & following tests.**Confirmation of presumptive colonies:**

- i. Germ tube test &
- ii. Fermentation and assimilation of sugars

Identification of *Aspergillus fumigatus*:

Colony characteristics & microscopic appearance

All culture media as well as biochemicals used were procured from HiMedia Laboratories, Mumbai, Maharashtra, India

All media, reagents & Antibiotic discs were procured from HiMedia Laboratories, Mumbai, Maharashtra, India

Quality control of culture media, Biochemicals & antibiotic discs was checked by standard methods prescribed.

*Jambhul Honey (Phondaghat pharmacy, Matunga Mumbai)

*Karvi Honey (Local market Mahabaleshwar) Honey samples were stored in dark at room temperature.

Honey samples were used as it is without any processing sterile distilled water was used as a diluent.

Sterility testing of Honey: The sterility testing of Honey was done by performing test for sterility as stated in the Indian Pharmacopeia²¹.

The *in vitro* antimicrobial activity of Honey was tested on isolates of *Klebsiella sp.*, *S. aureus.*, *P. aeruginosa* and *Candida* and *Aspergillus fumigatus* isolates. Standard ATCC strains of *E. coli* (ATCC 25922) *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *C. albicans* (ATCC 10231) were also tested along with the standard strains by Agar well diffusion method and Agar dilution method. Agar diffusion method was used as a screening method for determining the *in vitro* effect of test products on the test isolates and Agar dilution method was used to determine the minimum inhibitory Concentration of the test products.

• Preparation of different concentrations of Honey samples²²

Using sterile distilled water a 50% v/v solution of each honey was prepared taking all aseptic precautions. The 50% v/v solution of each Honey prepared was further diluted using distilled water to give a series of concentrations in the range of 5-50% v/v.

The antimicrobial activity of Honey was tested by standard microbiological techniques²³.

RESULTS AND DISCUSSION

In recent decades it is seen that bacterial infection factor has been overlooked in the casual treatment of bronchial asthma and allergies. But literature evidence suggests that bacterial infections play a major role in the etiopathogenesis of allergic diseases^{6,5}. Therefore along with treatment for allergic manifestations it is necessary to give treatment for these bacterial infections with wide spectrum antibiotics. This will result in cure in asthmatic patients without maintaining them on inhalers and unnecessary corticosteroid therapy.

In our study total 150 patients were selected. Out of 150 patients only 140 patients

could give satisfactory sputum samples, therefore remaining 10 patients were excluded from the study.

From 140 sputum samples tested for presence of microbial etiology, 32 (22.86%) isolates were obtained. 20 (14.29%) isolates were bacteria & 12 (8.57%) were fungi.

Total 20 bacterial isolates obtained were further identified as 10 isolates (50%) *Klebsiella pneumoniae*, 06 isolates (30%) *Staphylococcus aureus* & 04 isolates (20%) *Pseudomonas aeruginosa*. (Table 1) Similar findings are reported by Nagayama and Tsubaki T *et al*¹.

Table 1: Bacterial isolates obtained from sputum samples collected from patients with Asthma & ABPA

Total number of samples processed	Number of samples with bacterial isolate	Bacterial isolates		
		<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
140	20 (14.29%)	10 (50%)	06 (30%)	04 (20%)

Nagayama and Tsubaki *et al* cultured sputum specimens quantitatively from asthmatic children aged 0-14 years in order to determine the relationship between asthmatic status and bacterial species present in the respiratory tract¹.

In children with acute asthma attack, *H. influenzae*, *Streptococcus pneumoniae*, *M. catarrhalis* were distributed evenly in the samples. In patients with prolonged asthma attack pathogenic bacteria were present in 34.7% of patients. In these patients *H. influenzae* was predominant. In patients with pneumonia without asthma attack 40.9% of pathogenic bacteria were obtained. This study showed that there is a significant relationship between the presence of bacteria in sputum and clinical symptoms such as fever and pneumonia episodes during acute asthma attacks¹.

Several species and genera have been reported to cause fungal allergy. Epidemiological, environmental and clinical research was focused on relevant species like *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. Some studies reported the clinical relevance of *Candida*, *Trichophyton* and

Malssezia in either respiratory or skin allergic diseases. Allergy to spores of *Basidiomycetes* (e.g. *Boletus*, *Coprinus*, *Pleorotus*, *Psilocybes*) has been reported and the relevance of their causative role in respiratory allergy has been documented²⁴.

Sensitivity to fungal allergens has also been found to be a risk factor for severe life-threatening asthma. A New Zealand study of patients admitted to Hospital intensive care unit revealed that patients admitted to the ICU had a significantly greater incidence of reactivity to *Alternaria tenuis*, *Cladosporium cladosporoides*, *Helminthosporium maydis* or *Epicoccum nigrum*. Fungal cultures were performed from bronchial secretions of 13 asthma patients and from the skin of 91 patients with atopic dermatitis. The predominant yeast species present on the skin were *Candida* and *Rhodotorula* species, while *Candida species* were most predominant species isolated from bronchial secretions²⁵.

In the present study 12 (8.57%) fungal isolates were obtained. Out of total fungal isolates obtained 8 (66.67%) were *Candida* species, where 07 were *Candida albicans* & 01 was *Candida tropicalis*. (Table 2)

Table 2: Fungal isolates obtained from sputum samples collected from patients with Asthma & ABPA

Total number of samples processed	Number of samples with fungal isolates	Fungal isolates	
		<i>Candida species</i>	<i>A. fumigatus</i>
140	12 (8.57%)	08 (66.67%)	04(33.33%)

It is suggested that the yeast is an important causative allergen in bronchial asthma, rhinitis, chronic urticaria, atopic dermatitis, recurrent vaginitis and balanitis. In 1951 Keeny first reported asthma due to the yeast form of *Candida albicans*²⁶.

Therefore it is recommended that while treating allergic patients presence of *Candida species* in respiratory tract should be considered and focused treatment to irradiate *C. albicans* should be given so that *C. albicans* is cleared from respiratory tract otherwise as it is a well known fact that steroid therapy suppresses the immune system and fungal emergence as most severe in such cases.

Allergic Bronchopulmonary Aspergillosis (ABPA) is the most frequently recognized manifestation of allergic aspergillosis. It is an indolent disease with a protracted course, occurs worldwide and is now seen as an important emerging disease in India^{9,27}.

As the presence of asthma is the usual feature of ABPA, some of the asthmatics may become potential candidates for ABPA after the onset of asthma and hence require long term follow-up. With the employment of highly sensitive technique like ELISA, antibodies to *Aspergillus* may be detected in more number of cases in this specific group of patients²⁸.

In 1968 Henderson et al reported that, of their 46 asthmatic patients, 11% had definite allergic aspergillosis²⁹.

All these studies give a strong support to the importance of fungal sensitization as a important risk factor for the increasing severity of asthma. Exposure to environmental molds may play a role in asthma –related mortality²⁴. *Aspergillus fumigatus* was the only *Aspergillus* species isolated in the present study. 4 *Aspergillus fumigatus* isolates were obtained (33.33%).

In view of susceptibility of asthmatics and critically ill patients for allergic sensitization to *Aspergillus* antigens, it is necessary to consider this aspect in diagnosis, treatment and follow-up of these patients in order to avoid further complications associated with this. The emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to the public health.

Extensive use of cotrimoxazole, erythromycin and other antimicrobials in restricted areas has led to emergence of strains resistant to these antibiotics³⁰.

The frequency of serious fungal infections is also rising and this might be due to factors such as the increasing use of cytotoxic and immunosuppressive drugs to treat both malignant and non-malignant diseases. the increasing prevalence of infection due to human immunodeficiency type I and the widespread use of newer and more powerful antibacterial agents³¹.

In order to control the rise in antibiotic resistance and conserve the activity of current agents, the volume of antibiotics to which bacteria are exposed should be reduced³². It is seen that the emergence of microbial strains with multiple patterns of antimicrobial resistance has reduced the efficiency of conventional therapies.

Honey has been used as a vehicle for faster absorption of various drugs such as herbal extracts. Honey is also used as a vehicle for Ayurvedic preparations³³.

In the present study, we studied *in vitro* antimicrobial effect of natural products like Honey, on clinical isolates isolated from sputum patients with respiratory allergies. ATCC strains and clinical isolates of *P. aruginosa*, *S. aureus* and *Candida albicans* and *Aspergillus fumigatus* were studied.

Although these products are effectively used as a home remedy, the scientific literature to support their use is very less.

In the present study we studied antimicrobial effect of two Indian honeys, Jambhul Honey and Karvi Honey. Jambhul Honey was procured from Phondaghat Pharmacy and Karvi Honey was procured from local market of Mahabaleshwar.

These two honeys were studied for their physicochemical properties and sterility. The antimicrobial activity of these honeys were studied by Agar well diffusion and Agar dilution method against standard ATCC strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. fumigatus* as per CLSI guidelines²³.

In the present study Jambhul Honey and Karvi Honey were found to be sterile. Amongst all documented studies regarding antibacterial activity of honey Subrahmanyam et al considered the aspect of sterility of honey and he found honey of the plant source cumini (Jambhul) to be sterile³⁴.

Jambhul honey was dark brown in colour and Karvi Honey was brown in colour. The pH of the honeys tested was found to be 3.2 for Jambhul Honey and 2.9 for Karvi Honey.

Amongst all the organisms tested, majority were sensitive to 50% concentration of Jambhul and Karvi honey. Jambhul Honey was found to possess more antimicrobial activity than Karvi Honey.

Maximum zone of inhibition were shown by 50% concentration of both the honeys, with antibacterial activity to all the organisms tested. It was observed that size of zone of inhibition increases with the increase in the concentration of honey. It was reported by Molan et al that effectiveness of honey as antibacterial agents increases with the decrease in the concentration of honey, especially in Manuka and Pasteur honey from New Zealand^{35,36}.

For all concentration of Jambhul Honey, & Karvi Honey, average zone of inhibition was observed maximum with ATCC strains tested followed by clinical isolates.

Table 3: Agar diffusion method for Jambhul & Karvi Honey

Organisms tested	Results obtained in agar diffusion for Jambhul & Karvi Honey			
	Jambhul Honey		Karvi Honey	
	Range of Honey concentration in which zones of inhibition observed	Average zone of inhibition diameter	Range of Honey concentration in which zones of inhibition observed	Average zone of inhibition diameter
<i>E. coli</i> ATCC	5 -50%	29.00-42.00mm	10-50%	22.35-34.8 mm
<i>Klebsiella pneumoniae</i>	5-50%.	23.15 -40.15mm	10-50%	13.38-24.73mm.
<i>S. aureus</i> ATCC	5-50%	26.8 – 45.5 mm	5 -50%.	24.5-40.1 mm
<i>S. aureus</i>	5-50%	24.11-42.17 mm	5-50%.	23.47-38.2 mm
<i>P. aeruginosa</i> ATCC	40-50%	22.1-27.00 mm	40-50%.	20.0-24.0mm
<i>P. aeruginosa</i>	40-50%	20.1-23.00 mm	40-50%.	14.97-21.38mm
<i>C. albicans</i> ATCC	35-50%	15.9-22.0mm	35-50%	9.35-21.3 mm
<i>Candida species</i>	35-50%	13.3-20.61mm	35-50%	8.5-19.35 mm
<i>Aspergillus fumigatus</i>	40-50%	10.76 – 12.9 mm	40-50%	9.23 – 11.00 mm.

E. coli ATCC, *S. aureus* ATCC, *K. pneumoniae* showed zone of inhibition in the range 5-50% concentration for Jambhul Honey, with average zone diameter in range of 23.15-42.00 mm for *Klebsiella pneumoniae* & 24.11-42.17 mm for *S. aureus*.

P. aeruginosa ATCC & Clinical isolates showed zone of inhibition in range 40-50% concentration for Jambhul Honey with average

zone of inhibition 22.1-27.00 mm for Standard strain & 20.1-23.00 mm for clinical isolates of *P. aeruginosa*.

C. albicans ATCC & *C. albicans* clinical isolates showed zone of inhibition in range 35-50% concentration of Jambhul Honey with average zone of inhibition 15.9-22.0 mm for Standard strain & 13.3-20.61 mm for clinical isolates.

A. fumigatus isolates showed zone of inhibition in the range 40-50% concentration of Jambhul honey with average zone of inhibition 10.76 to 12.9 mm

Similar results were obtained with Karvi Honey. The zones of inhibition obtained for Karvi Honey were less as compared with Jambhul Honey & Concentrations of Karvi Honey showing zone of inhibition were more in case of *E. coli* ATCC strain & *K. pneumoniae*. (Refer table no. 3)

In the present study comparing the zones of inhibition for various concentrations of the honeys, to all the organisms it was observed that *S. aureus* was the most sensitive organisms to honey.

Our findings agree with Willix *et al*³⁷ who reported that *S. aureus* as the most sensitive organism to honey.

It is observed that *S. aureus* has developed resistance to many antibiotics especially emergence of MRSA. But as it can be seen from our observations that honey is very effective remedy for treatment of respiratory infections due to *S. aureus*.

Followed by *S. aureus*, *Klebsiella spp* and *P. aeruginosa* showed zones of inhibitions to various concentrations of both the honeys.

Zones of inhibitions shown by *P. aeruginosa* were less as compared to other organisms, showing that *P. aeruginosa* is most resistant organism to honey. This results collaborates with the findings reported by Andargarchew *et al*^{22,38}.

Both the honeys also showed *in vitro* effectiveness against *Candida* and *Aspergillus* isolates tested. *Candida* isolates were more susceptible to both the honeys more than *Aspergillus* isolates. Maximum zone of inhibition was obtained with 50% concentration of honey.

Agar dilution method was performed in order to determine the MIC of these honeys towards the organisms tested, but complete inhibition was not observed in case of any organism tested. There was reduction in the amount of growth indicating that honey may have acted as static agent rather than cidal for the isolates tested.

In order to determine the effectiveness of these honeys in treating respiratory infections along with *in vitro* studies and *in vivo* animal studies are necessary to be carried out.

As honey is a natural product, the compositions of honey are highly variable. The variation in sensitivity is also attributed to differences in growth rate of pathogens, nutritional requirements, temperature, inoculum size and the test medium used. It has been demonstrated that all honeys do not have same degree of antimicrobial activity.

When agar dilution method was performed it was observed that both honeys exhibit bacteriostatic effect rather than bactericidal.

CONCLUSION

Honey showed excellent *in vitro* antimicrobial activity.

Amongst the two Honeys tested Jambhul Honey was found better than Karvi Honey in their antimicrobial activity. Both these Honeys are Indian Honeys. This again emphasizes on medicinal value of traditional Indian products.

Both Jambhul & Karvi Honey gave bacteriostatic effect. Use of Honey has been already documented as a vehicle for other drugs as well as it has role in controlling infections. Garlic is also used as home remedy. In allergic respiratory infections presence of pathogens in respiratory tract increases severity of symptoms as well as duration of disease.

As it is observed that Honey has antimicrobial activity over a wide range of dilutions against pathogens isolated from patients having allergic respiratory infections, these agents can be included in routine treatment of such conditions along with antibiotics or as a single agent for therapy. This will help in cure of patients with no or minimum use of antibiotics which will give relief to patient as well as will prevent emergence of drug resistant organisms.

Acknowledgements

Authors are thankful to Dr. S. T. Nabar & Dr. Sangeeta Pednekar, Department of Medicine,

& Dr. Jayanthi Shastri, Head Microbiology, B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

REFERENCES

- 1 Nagayama, Y., Tsubaki, T., Role of bacterial infection in the exacerbation of acute or prolonged asthma attack in children *Allergology International*, June **48(2)**: pp 137-144 (8) (1999).
- 2 Sebastian, L., Johnston and Richard, J., Martin, *Chlamydophila pneumoniae and Mycoplasma pneumoniae: A Role in Asthma Pathogenesis?* Am J Respir Crit Care Med **172**: pp 1078 -1089 (2005).
- 3 Verghese, A., Immune Profile in Respiratory Viral Infections in Asthmatics *Indian J. Allergy Immunolo* **15(2)**: 87-92 (2001).
- 4 Tarfe, K., Deshpande, S., Bacterial etiology: significant in Allergic Respiratory Diseases, *Int. J. Curr. Microbiolo. Appl. Sci* **5(6)**: 577-587 (2016).
- 5 Ekiel, A., Friedek, D., Szulakowski, Romanik, M., Incidence of selected bacterial pathogens of the respiratory tract in patients with bronchial asthma *Wiad Lek* **55(1-2)**: 11-8 (2005).
- 6 Oehling, A. K., Bacterial infection as an important triggering factor in bronchial asthma, J., *Investg. Allergol Clin Immunol.* Jan-Feb; **9(1)**: 6 -13 (1999).
- 7 Gehring, U., Heinrich, J., Hoek, G., Giovannangelo, M., Bacteria and mould components in house dust and children's allergic sensitization *Eur Respir J* **29**: 1144-1153 (2007).
- 8 David, H., Broide Role of Bacterial infections in Allergy: A clinical Paradox Review from 57th Annual Meeting of the American Academy of Allergy, *Asthma and Immunology*, March 16-21, New Orleans, Louisiana (2001).
- 9 Maurya, V., Gunani, H. C., Sensitization to Aspergillus antigens and Occurrence of Allergic Bronchopulmonary Aspergillosis in Patients with Asthma *Chest* **127**: 1252-1259 (2005).
- 10 Gupta, P. R., Mangal, D. K., Prevalence of risk factors for bronchial asthma in adults in Jaipur district of Rajasthan *Lung India* **23**: 53-58 (2006).
- 11 White, J. W., Honey : a comprehensive survey, Edited by E. Crane London, Heinemann
- 12 Krell, R., Value added products from beekeeping FAO, Agricultural services, Bulletin 24
- 13 Deshpande, S. D., Kambli, K. S., Gawde, B. L., Honey: A potent agent for wound treatment, *The Journal of General Medicine* April-June **19(2)**: 42-46 (2007).
- 14 Elmer, W., Allen, K. S. D., William, M., Janda In Colour Atlas and Textbook of diagnostic Microbiology, 14th edition, *J. B. Lippincott Company* (1992).
- 15 Henry, D., Isenberg Clinical Microbiology Procedures Handbook, I, American Society for Microbiology (1992).
- 16 Revised National Tuberculosis Control Programme (RNTCP) Manual for Laboratory Technicians Central TB division, Directorate General of Health services, Ministry of health and Family Welfare, Nirmal Bhavan, New Delhi, May (1999).
- 17 Bailey and Scott's Diagnostic Microbiology 11th edition Alice Weissfeld, Mosby, (2002).
- 18 Blasi, F., Damato, S., Cosentini, R., *Chlamydia pneumoniae* and chronic bronchitis: association with severity and bacterial clearance following treatment *Thorax* **57**: 672-676 (2002).
- 19 S Frankel, S., Reitman, S., Sonnenwirth, A. C., Gradwohl's Clinical Laboratory methods and diagnosis: 7th edition: I and II: C. V. Mosby Company (1970).
- 20 Larone, D. V., Medically Important Fungi: A guide to identification: 1st edition: Harper Rou (1976).
- 21 Indian Pharmacopoeia II, S Appendix **9**: A-100 (1996).
- 22 Andargarchew, M., Tessemal, B., Debie, F., *In vitro* assessment of the antimicrobial potential of honey on

- common human pathogens Ethiop. J. Health Dev. **18(2)**: 107-111 (2004).
- 23 S Reeves, D. S., Philips, I., Williams, J. D. and Wise, R., Laboratory methods in antimicrobial chemotherapy: Churchill Livingstone (1978).
- 24 Mari, A., Schneider, P., Wally, V., Breitenbach, M. and Simon, B., Nobbe Sensitization to fungi: Epidemiology, Comparative skin tests and IgE reactivity of fungal extracts Clin Exp Allergy **33**: 1429-1438 (2003).
- 25 Alan, L., Miller The Etiologies, Pathophysiology and Alternative / Complementary Treatment of Asthma Alternative Medical Review February (2001).
- 26 Paula, S. A., Moraes Recurrent vaginal candidiasis and allergic rhinitis: a common association. *Annals of Allergy, Asthma and Immunology* **81**: 165 – 169 (1998).
- 27 Jindal, S. K., Respiratory Disease Epidemiology In India Lung India **23**: 93-94 (2006).
- 28 Kumar, A. A., Sahu, R. C., Subbannarayyar, K. K., Rau, P. V., Prevalence of antibodies to Aspergillii in bronchial asthmatics J of Postgraduate Medicine **35(1)**: 20-3 (1989).
- 29 Henderson, A. H., Englksih, M. P. and Veeht, R. J., Pulmonary Aspergillosis: A survey of it's occurrence in patients with chronic disease and a discussion of the significance of diagnostic tests Thorax **23**: 513-518 (1968).
- 30 Reba Kanungo, A., Kumar, S., Bhattacharya Antimicrobial Resistance among common bacterial respiratory pathogens *Indian Journal of Medical Microbiology* **18(2)**: 55-61 (2000).
- 31 Rex, J. H., faller, M. P., Rinaldi, M., Antifungal susceptibility testing Clinical Microbiology Reviews 367-381 (1993).
- 32 Wilson Bacteria, R., antibiotics and COPD Eur. Resp. J. **17**: 995-1007 (2001).
- 33 Nadkarni's, K. M., Indian Materia Medica with Ayurvedic, Unani –Tibbi, Siddha, Allopathic, Homeopathic Naturopathic and Home remedies, Appendices and Indices II Bombay popular prakashan (1976).
- 34 Subrahmanyam, M., Hemady, A. R., Pawar, S. G., The sensitivity of Honey to multidrug resistant *P. aeruginosa* from infected burns Annals of Burns and Fire disasters **16(2)**: (2003).
- 35 Cooper, R. A., Halas, E., Molan, P. C., The efficacy of honey in inhibiting strains of *P. aeruginosa* from infected burns J. Burn Care and Raehabilitation 366-370 (2002).
- 36 Cooper, R. A., Molan, P. C. and Harding, K. G., The sensitivity of honey to Gram positive cocci of clinical significance isolated from wounds J. Applied Microbiology **93**: 857-863 (2002).
- 37 Wilix, D. J., Molan, P. C., Harfoot, C. J., A comparison of the sensitivity of wound infecting species of bacteria to the antimicrobial activity of Manuka honey and other honey J Appl Bacteriology **73**: 388-394 (1992).
- 38 Kariyal, S., Deshpande, S. D., Koppikar, G. V., Effect of honey on multidrug resistant organisms & it'ssynergistic action with three common antibiotics Jr. of Postgraduate Medicine Oct. –Dec. **44(IV)**: 93-96 (1998).