

***In vitro* Assessment of Antipseudomonal Activity of Honey and Citric Acid**

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The present study was aimed to investigate the efficacy of honey and citric acid against multidrug resistant clinical isolates of *Pseudomonas aeruginosa* from infected wounds. A total of twenty-four isolates of *Pseudomonas aeruginosa* were studied for sensitivity to honey and citric acid on the principle of minimum inhibitory concentration (MIC). The antibiogram suggests that all *Pseudomonas aeruginosa* isolates were resistant to Gentamicin and susceptible to Ciprofloxacin. The order of antibiotic resistance in *Pseudomonas aeruginosa* noted was Polymyxin-B > Ceftizoxime > Piperacillin > Tobramycin > Carbenicillin > Ceftazidime > Norfloxacin > Cefepime. The majority (80%) of multidrug resistant *Pseudomonas aeruginosa* isolates were inhibited by 30 % v/v and 500 micrograms/milliliter of Honey and Citric acid concentration respectively. The present study showed that *Pseudomonas aeruginosa* isolates resistant to routinely used antibiotics were sensitive to antibacterial action of honey and citric acid. The findings of the present study demonstrated the role of honey and citric acid as antipseudomonal agents. This suggests that antibacterial honey and citric acid have potential to be an effective alternative antibacterial agent.

Key words: *Pseudomonas aeruginosa*, Wound infection, Honey, Citric acid.

Pseudomonas aeruginosa is clinically significant due to the fact that it is an etiological agent in number of infections such as septic burns and wounds, UTI, conjunctivitis, meningitis, etc. It serves as a reference species in antimicrobial susceptibility testing on account of its notorious resistance to several antimicrobial compounds. The wound is an ideal substance for bacterial growth. It provides a wide portal for microbial invasion from the surrounding skin and the hospital environment. Despite of recent advances in antimicrobial chemotherapy and wound management, infection continues to be an important problem.

The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature (pH being 3.2-4.5), hydrogen peroxide concentration and its phytochemical nature, i.e. its content of tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, flavonoides, streptomycin, sulfathiazole, trepens, benzyl alcohol, and benzoic acids^{1,2}. Honey is one of the oldest traditional medicine considered to be important in the treatment of infections especially where a conventional treatment has failed.

There have been many reports published of the sensitivity to honey of a wide range of bacterial species, and some of these studies have included *Pseudomonas aeruginosa*¹.

A variety of chemical agents are available, which are nontoxic, inexpensive and highly effective against various organisms. These agents can be used in the treatment of wound infections

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and the use of antibiotics can be avoided to some extent. Use of citric acid has also been reported in the treatment of a variety of wounds infected with various bacteria in human beings^{3,4,5}.

The aim of the present study was to investigate the efficacy of Honey and Citric acid in inhibiting strains of *Pseudomonas aeruginosa* isolated from infected wounds.

MATERIALS AND METHODS

Isolation and Identification

The present study was carried out in P.G.Department of Microbiology, Dhote Bandhu Science College, Gondia (Maharashtra). Ingredients of bacteriological culture media and chemicals were obtained from HiMedia Laboratories Pvt, Limited, Mumbai. All the chemicals used were of analytical grade. The samples were collected by swabbing sterile cotton swabs over wounds of patients. A total of twenty swabs of pus sample were collected from patients. The individual samples were inoculated onto Cetrimide Agar Plates separately and plates were incubated at 37°C for 24 hours. The colonies with typical blue green pigmentation were designated as *Pseudomonas species* and pure culture were obtained by sub-culturing. The isolates were identified by conventional tests such as Gram staining, Motility test, Catalase test, H₂S production test, Urea hydrolysis test, IMViC test, Nitrate reduction test, Oxidase test, O/F test and sugar fermentation profile⁶. The isolates were maintained on nutrient agar slants and stored at 4°C. A total of twenty four isolates were selected for further study.

Antibiotic susceptibility test

The antibiotic sensitivity of organism was performed on Muller Hinton Agar plates by Kirby-Bauer Disk Diffusion test [7]. The commercially available antimicrobial discs used for susceptibility test were Norfloxacin (10 mcg), Polymyxin-B (300 units), Ciprofloxacin (5 mcg), Gentamycin (10 mcg), Piperacillin (100 mcg), Tobramycin (10 mcg), Carbenicillin (100 mcg), Cefepime (30 mcg), Ceftazidime (30 mcg), and Ceftizoxime (30 mcg). All the isolates were inoculated into nutrient broth (10 ml) and incubated for 24 hours at 37°C, the inoculum density was made equivalent to 0.5 McFarland standard. The size of

zone of inhibition was measured by using metric ruler which was held on the back of the inverted Petri plate. The zone diameters of each antibiotic are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS)⁸.

Determination of MIC of honey by broth dilution method

The test was carried out as described by Heuvelink *et al.*⁹ with some modifications. In this procedure, different concentrations of honey (% v/v) were prepared by using Mueller-Hinton broth in order to have final concentrations as 10%, 20%, 30%, 40%, 50 % and 60% v/v. Each tube was inoculated with 0.1 ml of 0.5 McFarland standard equivalent inoculum of test organism. The inoculated tubes were incubated at 37°C for 24 hours. Tubes were then examined for visible signs of bacterial growth. The first tube showing macroscopic inhibition of growth is considered as the minimum inhibitory concentration (MIC). The lowest concentration (highest dilution) of honey preventing appearance of turbidity is considered to be the minimum inhibitory concentration.

Determination of MIC of citric acid by broth dilution method

The test was performed as described by Baron *et al.*,¹⁰ with some modifications. In this procedure, different concentration of citric acid (micrograms/milliliters) were prepared by using Mueller Hinton broth in order to give its final concentration 100,200,300,400,500,600, and 700 micrograms/milliliter. Each tube was inoculated with 0.1 of 0.5 McFarland standard equivalent inoculum of test organism. The inoculated tubes were incubated at 37°C for 24 hours. Tubes were then examined for visible signs of bacterial growth. The first tube showing macroscopic inhibition of growth is considered as the minimum inhibitory concentration (MIC). The lowest concentration (highest dilution) of citric acid preventing appearance of turbidity is considered to be the minimum inhibitory concentration.

RESULTS AND DISCUSSION

Isolation and identification

Out of twenty pus samples tested for the presence of *Pseudomonas aeruginosa*, twelve

samples (60%) were found *Pseudomonas aeruginosa* positive. These isolates showed typical colonies on cetrimide agar. They were Gram negative and motile. They showed Citrate, Oxidase, Catalase, and nitrate positive while indole, methyl red, and vogues proskauer and urea hydrolysis test negative. They produced H₂S (slant and butt-alkaline red). They did not ferment lactose, maltose and mannitol but ferment glucose to produce acid only aerobically. Mac-Conkey agar plate showed colorless non lactose-fermenting colonies. They showed typical *Pseudomonas aeruginosa* colonies on Pseudomonas isolation agar.

Antibiotic susceptibility test

The resistance profile of twenty-four *Pseudomonas aeruginosa* isolates from pus to commonly used antibiotics is tabulated in Table:

1. Table:1. Resistance profile of *Pseudomonas aeruginosa* isolates (n=24) to the commonly used antibiotics.

Table 1. Antibiotic resistance profile of *Pseudomonas aeruginosa* isolates (n=24)

S.No	Antibiotic	% Resistance
1	Ciprofloxacin (5 mcg)	0.00%
2	Cefepime (30 mcg)	20.84
3	Norfloxacin(10 mcg)	29.1%
4	Ceftazidime (30 mcg)	37.50%
5	Carbenicillin (100mcg)	41.67%
6.	Tobramycin (10 mcg)	54.17%
7.	Piperacillin (100 mcg)	54.17%
8.	Ceftizoxime (30 mcg)	75.00%
9.	Polymyxin-B (300 units)	91.70%
10.	Gentamycin (10 mcg)	100%

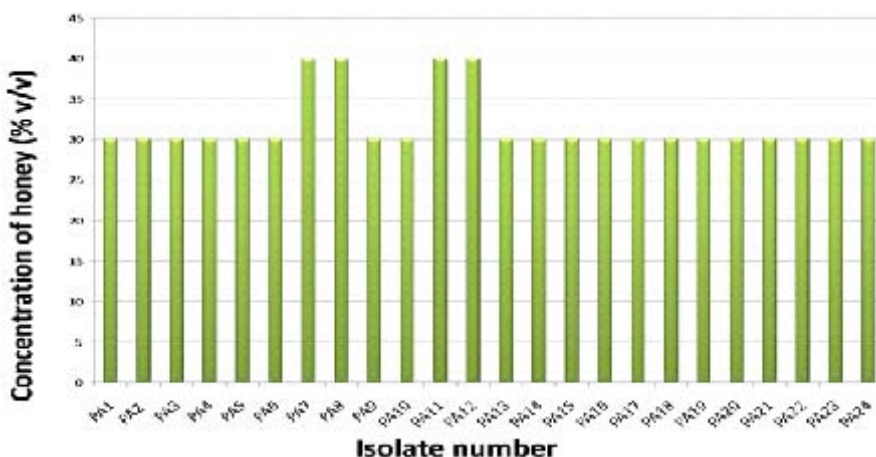


Fig. 1. Sensitivity to honey of *Ps. aeruginosa* isolates

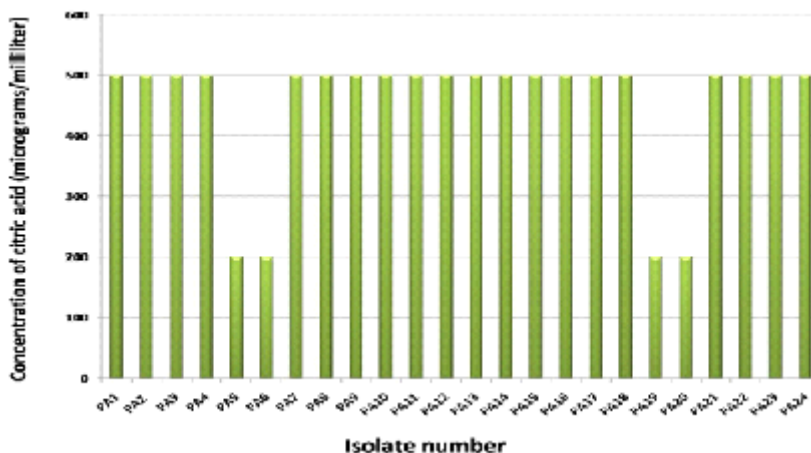


Fig. 2. Sensitivity to citric acid of *Ps. aeruginosa* isolates

In the present study, *Pseudomonas aeruginosa* isolates showed multiple antibiotic resistance. All the *Pseudomonas aeruginosa* strains were found to be susceptible to ciprofloxacin and resistant to gentamycin. Similar to our study drug resistance has been observed by Subrahmanyam et al (2003). In their investigation, fourty-four isolates of *Pseudomonas aeruginosa* were characterized and they reported resistance of *Pseudomonas aeruginosa* to Erythromycin, Ampicillin, Gentamycin, Penicillin, Kanamycin, Amoxicillin, Ciprofloxacin and Norfloxacin¹¹.

Antibacterial power of Honey:

In our study, the MIC for honey against *Pseudomonas aeruginosa* reported was 30 & 40 % v/v (Fig. 1).

In similar studies of R.A.Cooper, et.al (2002), they noted MIC for honey below 10 % v/v against *Pseudomonas aeruginosa*¹²

Antibacterial power of citric acid

In this study twenty multidrug resistant strains of *Pseudomonas aeruginosa* were inhibited at citrate concentration of 500 micrograms/milliliter (Fig. 2).

Similar to our study, Wadher B.J et al (2006) and Nagoba B.S et al (2008) reported MIC for citric acid 1000 micrograms/milliliter and 900 micrograms/milliliters for *Pseudomonas aeruginosa* and *S. aureus* respectively^{13,4}.

CONCLUSION

Our data express *Pseudomonas aeruginosa* is highly prevalent in the wound infection. The antibiotic resistance of isolates in this study appears to suggest a need for continuous monitoring of bacterial antibiotic susceptibility testing for antibiotic prescription in order to ensure adequate treatment of wound infection.

Honey & citric acid have great potential as antimicrobial agents against *Pseudomonas aeruginosa*. This findings from the present study indicate that honey and citric acid with antipseudomonal activity has the potential to be a very useful treatment option for infected wounds.

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