Synergistic effect of honey in combination with silver nanoparticles on isolated pathogens from urinary tract infection

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Background

Urinary tract infections (UTIs) are the most prevalent bacterial infections, affecting 150 million people worldwide each year. UTIs can be caused by a variety of pathogens, but *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis* are the most frequent culprits. The growth and development of antibiotic-resistant bacteria are currently a major worry for the international health community. Looking for a treatment substitute could be effective in tackling this issue. The honey has a low pH (mean 4.4) value, which makes it unfavorable for bacterial growth; undiluted honey combination with silver nanoparticles (AgNPs) shows synergistic antimicrobial effect and helps lessen infection.

Aim

This study was undertaken to find out the effectiveness of honey containing AgNPs in the antibacterial activity of isolated urine pathogens.

Patients and methods

A total of 30 patients with UTI were included through purposive sampling technique, and urine samples were collected from them. A semiquantitative approach was used to cultivate specimens of urine on MacConkey agar and blood agar. After 24 h of incubation, bacterial growth was seen on the plates. Then, colonies were counted and the number of microorganisms per milliliter in the original material was calculated by multiplying the number of CFUs by 1000.

Results

A total of 10 bacterial isolates were found in the 30 urine samples, and biochemical studies showed that these isolates were from three different species. The most common strain of *E. coli* represented 82%, followed by *P. aeruginosa* (12%) and *P. mirabilis* (6%). In all three organisms examined with undiluted honey mixed with AgNPs, the zone of inhibition was more pronounced when compared with only honey, AgNPs, and diluted honey mixed with AgNPs.

Conclusion

The results of the current investigation showed that multifloral honey combined with AgNPs was effective against urinary infections.

Keywords:
honey, silver nanoparticles, urinary tract infection

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Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, affecting 150 million people worldwide each year [1]. They are caused by a variety of pathogens, but *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *P. mirabilis* are the most frequent culprits [2]. On the contrary, several resistance genes and gene products work in concert with plasmid addiction, efflux, and hypermutability to accelerate the growth of the bacterial population. Such criteria indicate that all or the majority of the medications in a given therapeutics are impaired. Therefore, UTIs brought on by resistant bacteria will have higher effects [3]. Therefore, compared with infections caused by susceptible organisms, UTIs produced by resistant organisms have higher morbidity and mortality, are more expensive to treat, lead to longer hospital admissions, and are a greater strain on health care systems. In the case of a simple urinary infection, nitrofurans and trimethoprim are the antibiotics of choice, whereas fosfomycin and lactam antibiotics come in second. In outpatient practice, fluoroquinolones are typically used to treat complex UTIs [4], although recent research has shown that drug resistance to these substances is rising [5]. The growth

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and development of antibiotic-resistant bacteria are currently a major worry for the international health community. To resolve this problem, looking for a treatment substitute could be effective. Traditional medicine has a long history of using natural products to address a variety of health issues, including infections. Honey, as one of the most well-known and powerful antibacterial agents, has a wide range of activity against both gram-positive and gram-negative bacteria. Its nutritional, therapeutic, and antibacterial benefits are supported by a substantial body of scientific research. Approximately 200 different substances – amino acids, vitamins, minerals, and enzymes – are found in bee honey, although sugar and water make up the majority. The two primary carbohydrates in honey are glucose and fructose [6]. Bee honey has the potential to be an antibacterial agent as it has inhibitory effects on about 60 different bacterial species, including aerobes and anaerobes, as well as gram-positive and gram-negative bacteria [7]. There are a number of variables that contribute to honey's antimicrobial effectiveness, including its high sugar content, which prevents bacteria development. This is thought to be due to its osmotic effects, which inhibits bacterial growth and hence aids healing. Honey is hygroscopic, which means that it takes moisture from its surroundings and use its hyperosmolar qualities to dehydrate germs [8]. Honey has a low pH (mean 4.4) value, which makes it unfavorable for bacterial growth and helps lessen infection or wound colonization. Additionally, glucose oxidase, which continuously produces hydrogen peroxide, may be responsible for the antibacterial properties of bee honey. Hydrogen peroxide has the ability to interact with signals that bacteria use to proliferate, which inhibits bacterial growth even in honey that has been diluted. With no negative effects on the host tissues, honey offers anti-inflammatory, antioxidant, antithrombotic, anti-allergic, vasodilator, and wound healing capabilities. It has bacteriostatic and bactericidal properties because of its high osmolarity. Previous research has shown that honey is effective in treating wounds in which *S. aureus* and *P. aeruginosa* are present [9]. There are several uses for silver nanoparticles (AgNPs), and it has been observed that at low concentrations, AgNPs are most effective against bacteria, viruses, and other eukaryotic microorganisms while being nontoxic to humans [10]. Numerous studies have suggested that silver and AgNPs may bind to cell surface membranes, impairing permeability. Consequently, silver's antibacterial properties have been used in a number of medical applications [11]. AgNPs exhibit multiple and simultaneous mechanisms of action, and in combination with antibacterial agents as organic compounds or antibiotics, they have shown synergistic effects against pathogenic bacteria such as *E. coli* and *S. aureus*. The characteristics of AgNPs make them suitable for their application in medical and health care products, where they may treat infections or prevent them efficiently [12]. The current investigation was conducted to find out whether honey containing AgNPs can boost the antibacterial activity of isolated urine pathogens.

Patients and methods

Study setting

Specimens required for the study were obtained from the patient unit of a tertiary care hospital of a rural hospital in Andhra Pradesh.

Ethical consideration

Before beginning the study, the Institutional Ethics Committee gave its assent, with reference number 01/IEC/GEMS&H/2021. All of the study participants provided written informed consent.

Study population

To avoid contamination, the urine sample was properly collected in a sterile container. Through the purposive sampling method, 30 patients with UTI were used as study participants, and samples were collected from them. Sterilized and pasteurized multifloral honey was purchased from Bharat Unani Pharmacy in Hyderabad, India, to test for antibacterial activity. AgNPs were purchased from Hi-Q nanotechnology in Bangalore, Karnataka. A 5-g disc of ciprofloxacin was purchased from Himedia Laboratories Pvt Ltd in Mumbai, India.

Methodology

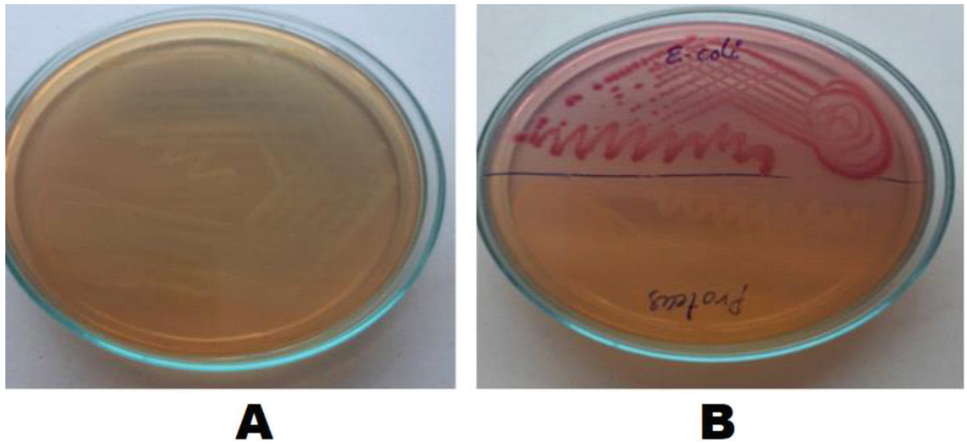
Urine culture

The semiquantitative approach was used to cultivate specimens of urine on MacConkey agar and blood agar. The urine sample was inoculated into the medium using the loop method. Urine was carefully blended before inoculation. This technique was used to produce a sterile loop (4 mm loop) that supplies 0.01-ml volume of urine on blood agar plate and MacConkey agar plate (Fig. 1). They were incubated for a minimum of 24 h at 37°C.

Interpretation of the culture

After 24 h of incubation, bacterial growth was seen on the plates. Colonies were counted and the number of microorganisms per milliliter in the original material

Figure 1



Bacterial culture growth of *Pseudomonas*, Escherichia *coli*, and *Proteus*.

was calculated by multiplying the number of CFUs by 1000.

Identification of isolates

The isolates were identified based on colony shape and biochemical characterization (Table 1) in blood agar and MacConkey agar [13,14]. For this investigation, the three most prevalent bacteria, *E. coli*, *P. mirabilis*, and *P. aeruginosa*, were isolated. These bacteria were identified using the preliminary and generally accepted laboratory techniques listed in Fig. 1.

Testing for antimicrobial susceptibility

According to the CLSI 2016 guidelines, the Kirby-Bauer disc diffusion method was used to evaluate all isolates susceptibility patterns and test them using the *in vitro* agar disc diffusion method. To create a grass culture, a sterile Mueller-Hinton agar (Hi medium, Mumbai, India) plate was spread with a 4–6-h incubated bacterial culture solution and compared with a standard 0.5 McFarland scale. It was dried for 30 min at 37°C in the incubator. Using No. 1 filter paper, 6-mm diameter discs were created. Overall, 100 of these discs were prepared and then placed in a small glass bottle, sterilized in a hot air oven at 160°C for 2 h, and blended with 50 μl of pasteurized honey combined with 50 μl of undiluted AgNPs [13,14]. The test sample includes 1 : 10, 1 : 120 serial dilutions of undiluted honey combined with AgNPs . 50 μl of only honey, and 50 μl of AgNPs alone and normal saline added on discs as a negative control and left overnight to ensure that all discs absorbed honey and AgNPs equally, and ciprofloxacin (5 g) was standard antibiotic discs prepared as a positive control. Seven discs were prepared in two plates. To enable the bacterial growth, the plates were once again incubated aerobically at 37°C overnight. After incubation, the antibacterial activity of each of the tested discs zone of inhibition was measured using a scale that was obtained from HiMedia. Each strain's sensitivity testing plates were created in three copies. The mean and SD of the results were calculated.

Table 1 Morphological and biochemical characterization of selected isolates

Test	*Escherichia coli*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
Gram's staining	Gram-negative bacilli	Gram-negative bacilli	Gram-negative bacilli
Motility by hanging drop method	Motile	Motile	Motile
Catalase test	Positive	Positive	Positive
Oxidase test	Negative	Negative	Positive
Nitrate reduction test	Nitrate reduced	Nitrate reduced	Nitrate reduced
Indole	Positive	Negative	Negative
Methyl red	Positive	Positive	Negative
Voges-Proskauer	Negative	Negative	Negative
Simmons citrate	Negative	Positive	Positive
Christensen's urease	Positive	Positive	Positive

Statistical analysis

Data were evaluated statistically using the SPSS, 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). To determine the importance of the difference, an analysis of variance was used. Significance of the statistics was judged at *P* value of 0.05.

Results

A total of 10 bacterial isolates were found in 30 urine samples, and biochemical studies showed that these

Table 2 Antibacterial activity of honey combined with silver nanoparticles against selected urinary isolates

Name of the organism	Honey combined with silver nanoparticles (undiluted)	Diluted with different concentrations of Honey combined with Silver nanoparticles (v/v) with diameter zone of inhibition (mm)		Silver nanoparticles alone	Honey alone	Ciprofloxacin (positive control)	Normal saline (negative control)
		1 : 10	1 : 20				
Escherichia coli	35.5±0.3	28.3±0.2	12.4±0.3	26.5±0.1	25.7±0.21	32.4±0.3	0
Pseudomonas aeruginosa	38.5±0.3	28.1±0.2	13.3±0.2	30.5±0.2	29.5±0.4	31.7±0.2	0
Proteus mirabilis	28.2±0.2	25.2±0.16	10.3±0.2	23.5±0.2	22.4±0.3	25.3±0.2	0

isolates are from three different species. The most common strain was *E. coli* (82%), followed by *P. aeruginosa* (12%) and *P. mirabilis* (6%). Zone of inhibition was indirectly proportional to the diluted honey, according to the results shown in Table 2. In all three organisms examined with honey mixed with AgNPs, the zone of inhibition was more pronounced. The honey and AgNPs together have a substantial antimicrobial effect. In tests using honey and AgNPs, the zones of inhibition for three bacterial isolates – *E. coli*, *P. aeruginosa*, and *P. mirabilis* – showed greater sensitivity at 35.5, 38.5, and 28.2 mm, respectively. When compared with undiluted honey coupled with AgNPs, the zone of inhibition in diameter (mm) of three isolates was 28.3 ±0.2, 28.1±0.2, and 25.2±0.1, and 12.4±0.3, 10.3±0.2, and 13.3±0.2 in 1 : 10 and 1 : 20 dilutions, respectively. When compared with 1 : 10 and 1 : 20 dilutions, honey by itself and AgNPs alone also shown greater sensitivity, but less sensitivity than honey mixed with AgNPs, where 25.7±0.21, 29.5±0.4, and 22.4±0.3, and 26.5±0.1, 30.5±0.2, and 23.5±0.2 are the zones of inhibition. When compared with the 1 : 10 and 1 : 20 dilutions of honey combined with AgNPs and honey and AgNPs alone, the standard antibiotic disc ciprofloxacin did not exhibit greater sensitivity than honey combined with AgNPs in its undiluted form. Three bacterial urinary pathogens were not inhibited by a zone of inhibition on a normal saline disc.

Discussion

Three of the most prevalent bacterial isolates were chosen for this investigation using standard, advised laboratory techniques. *E. coli*, *P. mirabilis*, and *P. aeruginosa* were identified using the following preliminary and biochemical assays. The current work demonstrates the zone of inhibition's synergistic antibacterial action when AgNPs and honey are coupled. In tests using honey and AgNPs, the zones of inhibition for three bacterial isolates – *E. coli*, *P. aeruginosa*, and *P. mirabilis* – showed greater sensitivity at 35.5, 38.5, and 28.2 mm, respectively. When compared with undiluted honey combined with AgNPs, honey alone, AgNPs itself alone, and diluted honey combined with AgNPs in 1 : 10 and 1 : 20 dilutions showed less sensitivity. Honey and AgNPs alone also displayed greater sensitivity when compared to 1 : 10 and 1 : 20 dilutions, but less sensitivity when compared to honey combined with AgNPs. Typical antibiotic disc ciprofloxacin was used as a positive control and demonstrated increased sensitivity when compared to honey paired with AgNPs at dilutions of 1 : 10 and 1 : 20 and honey alone, but not to undiluted honey combined with AgNPs. Negative control was a normal saline disc, which showed no zone of inhibition for three bacterial urine infections. UTIs, which afflict millions of individuals each year and are major health issues, can be classified as complicated or uncomplicated. Patients with physically normal urinary tracts as well as those with structurally or functionally aberrant tracts can develop severe UTIs (uncomplicated UTI). UTI pathogens require intricate interactions between several species, the environment, and a possible host. UTIs are caused by aerobic gram-negative organisms. *E. coli* is typically isolated in 80–90% of initial infections, which typically originate from the normal microbial ecology [15]. UTIs are thought to be a considerable burden on public health and have a negative effect on people's quality of life. Other UTI complications include pyelonephritis and bacteremia, both of which, if left untreated, can result in severe mortality and permanent kidney damage. Treatment options for any bacteriuria that occurs during pregnancy should take into account the safety of the mother and the fetus. Fosfomycin and lactamins are the second-line antibiotics for treating uncomplicated urine infections after nitrofurans and trimethoprim [16]. Fluoroquinolones are typically used to treat severe UTIs in outpatient settings. For both the mother and the fetus during pregnancy, these medications are thought to be efficacious and safe [4,17]. However, current research studies have shown that these medications are becoming less effective. The global health community is currently extremely concerned about the emergence and spread of bacteria that are resistant to antibiotics [18]. Scientists are looking for alternative, effective antimicrobial agents to combat antibiotic resistance. Traditional medicine has been used to naturally cure a variety of health issues (such as infections) since ancient times. Gram-positive bacteria were more resistant to honey samples used in their investigation than gram-negative bacteria. The reason could be that different varieties of honey with various antibacterial properties were used in their investigation. Detailed analyses and several studies on honey variations with potential antibacterial and therapeutic characteristics have been published [19].

According to certain reports, honey exhibits bacteriostatic and bactericidal effects on a variety of gram-positive and gram-negative bacteria [20]. UTI treatments have a lot of room for improvement because of nanotechnology. AgNPs have been demonstrated to have antimicrobial activity, and it has been established that their size and concentration determine how effective they are. Most examined strains exhibit bactericidal effects when nanoparticles with diameters ranging from 5 to 100 nm are present in solutions at a concentration of 40 g/m [21]. The peptidoglycan layer is a unique membrane component of bacterial species and not mammalian cells, and Kim *et al.* [22] explored the antibacterial mechanism of AgNPs for particular microbial species. It will be simpler and more specific to use AgNPs as an antibacterial agent if the antibacterial effect of AgNPs is associated with the peptidoglycan layer, and it has been suggested that the antimicrobial mechanism of AgNPs is related to the formation of free radicals and subsequent membrane damage brought on by free radicals. Free radicals, which are responsible for the antibacterial activity [22], may be produced from the surface of AgNPs. AgNPs may be able to improve the effectiveness of antibiotics against both susceptible and resistant bacteria, as well as reduce bacterial adherence during the early phases of biofilm development, according to a number of recent studies. To assess antibiofilm activity, a study by Mala *et al.* [23], impregnated urinary catheters with antibiotics (amikacin and nitrofurantoin) and a synergistic mixture of antibiotics and AgNPs (produced via a biological approach). According to the authors, functionalization with antibiotics only exhibited a 25% suppression of bacterial adherence, but the synergistic combination showed a 90% inhibition. Combining honey and AgNPs has a synergistic antibacterial action that reduces the likelihood of infection or wound colonization [24]. According to earlier studies, AgNPs have the potential to be antibacterial against bacteria including *E. coli*, *S. aureus*, *Staphylococcus epidermis*, *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Klebsiella mobilis*, and *K. pneumonia*. It has been demonstrated that AgNPs made using various synthetic techniques have potent antibacterial properties. The benefits of using nanotechnology in contemporary medicinal applications are well known, and they have been shown to be the most promising area for the creation of novel bactericidal drugs to treat bacterial infections.

Conclusion

The results of the current investigation showed that multifloral honey combined with AgNPs was effective against urinary infections. The study suggests additional in-depth research in this area to support the usage of these products for medical purposes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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