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Natural antibacterial remedy for respiratory tract infections

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ABSTRACT

Objective: To evaluate the antibacterial activity of Egyptian honey against bacteria causing respiratory tract infections.

Methods: Sputum and throat swab specimens were used, from which five bacterial species were isolated, namely, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Streptococcus pneumonia* were isolated, identified and grown on suitable media for further identification or confirmation. Different concentrations (100%, 75% and 25%) of honey and simulated honey solution were used for activity assay and estimation of minimum inhibitory concentration and minimum bactericidal concentration.

Results: All the tested bacterial isolates were completely susceptible to the 75% concentrations of honey and to the 100% concentration of the simulated honey solution. This may be due to the high osmotic pressure exerted by the high sugar content in both honey samples. Moderate susceptibility of the isolated bacteria to honey at 100% v/v concentration, and resistance to honey at 25% concentration and the 75% and 25% concentrations of simulated honey solution, indicated the presence of other antimicrobial components responsible for the activity other than the osmotic pressure. Therefore, it was suggested that honey showed distinguished antibacterial activities against the most common bacteria causing respiratory infections with varied sensitivity.

Conclusions: Honey, a non-toxic, nutritious, safe for human consumption and cheap natural antibacterial agent, should be globalized.

1. Introduction

The respiratory tract begins from the larynx and consists of the oropharynx and nasopharynx in addition to the sinuses, the middle ear and finally extends to the lungs. Infection of the respiratory tract is one of the commonest illness in the general population and results in significant morbidity [1]. Over 50 million deaths around the world are caused by respiratory tract infections, which are the main cause for clinic visits and antibiotics prescription. Poor immunity and malnutrition are the main causes for the high incidence of respiratory tract infections.

The increase of quality of life over the past 50 years is mainly due to the use of antibiotics as antimicrobial chemotherapy. However, antibiotic-resistant bacteria have become a challenging public health problem worldwide [2]. The reason may be due to the side effects accompanying antibiotics systemic administration, such as hypersensitivity reactions, kidney problems, liver problems and gastrointestinal upset.

Natural health remedies and supplements are undergoing extensive studies to overcome such bacterial resistance to antibiotics and to offer alternative natural antimicrobial agents with least adverse effects on human body [3,4].

Honey was first used as a food source since ancient times and then became an effective natural cure for certain infections, such as some respiratory diseases and for the healing of skin burns and wounds [5]. The therapeutic property of honey has received well recognition from the medical field [6]. The antimicrobial potency and medical applications of honey are tremendous as it has demonstrated inhibitory effects against a number of pathogenic bacteria [7].

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Several researches reported that honey has an inhibitory effect over 60 species of bacteria including aerobes, anaerobes, Gram-positives and Gram-negatives [8]. Other studies showed that honey has broad antibacterial, anti-inflammatory and antioxidant effects and plays a role in boosting the body immune system. Honey is also characterized by its least adverse effects on human body [9].

A different study reported the antifungal action of honey against some yeasts infections and species of *Aspergillus* and *Penicillium* [8], in addition to the common dermatophytes [10].

Honey not only serves as a cheap antimicrobial agent but also a full nutritional source. It consists of carbohydrates (fructose, glucose), amino acids, minerals (calcium, sodium, phosphorus, magnesium, silicon, iron, manganese, copper), organic acids, water, vitamins (A, B complex, C, D, E), enzymes (invertase, amylase), and antioxidants (pinocembrin, ascorbic acid, catalase, selenium) [11]. Phenolic acids and flavonoids were found to play an important role in the therapeutic capacity of honey [9].

This study aims to evaluate the antibacterial activity of Egyptian honey against bacteria causing respiratory tract infections.

2. Materials and methods

2.1. Bacterial strains

Sputum and throat swab specimens were used, from which five bacterial species were isolated, recognized with respiratory tract infections at a local diagnostic lab. Five bacterial species were isolated. For complete identification of the isolated bacteria, the samples were inoculated on blood agar, chocolate agar, MacConkey agar and cetrimide agar (Oxoid UK), and the plates were incubated at 37 °C for 24–48 h. Identification of the growing microorganisms was done by colony morphology. Pure colonies were sub-cultured on blood agar, nutrient agar and chocolate agar media. Further identification or confirmation was carried out using biochemical tests as recommended by Cheesebrough [12].

2.2. Preparation of test samples

Crude honey obtained from Sinai, Egypt was used as concentrated solution. For the diluted crude solution, 50 mL sterile volumetric flask was used, where the required amount of crude honey was added, then the volume was completed with sterile distilled water to make solutions of 75% and 25% dilutions.

For preparing the simulated honey solution, 38.4 g of fructose, 30.3 g of glucose, 1.3 g of sucrose, 8.6 g of maltose and 1.4 g of maltodextrin were dissolved in 17.2 mL of distilled water.

A dilution series with simulated honey concentrations (75% and 25%) together with the diluted crude honey solutions (75% and 25%), in addition to the undiluted solutions of both the crude honey and the simulated honey, were used for the activity assays. Control plates of nutrient agar with no honey were made in duplicate and included in each susceptibility assay to confirm the viability and density of the cultures.

2.3. Antibacterial activity assay

Mueller-Hinton agar (Oxoid UK) was prepared for *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Klebsiella pneumonia* (*K. pneumonia*), and blood agar was prepared for *Streptococcus pyogenes* (*S. pyogenes*) and *Streptococcus pneumoniae* (*S. pneumoniae*). An autoclave at 120 °C was used to sterilize all media. Thirty milliliters of the agar media with the respective inoculated strains of bacteria were transferred aseptically in each sterilized Petri plate. All plates were left at room temperature to solidify. Wells of 6 mm diameter were made in the agar using a sterile cork borer. The test sample was placed in each respective well using sterile droppers. Antibacterial assay plates were incubated at 37 °C for 48 h. The exact procedure was also done in control plates, but instead the wells were filled with sterile distilled water for negative control and the standard antibiotics disc of 6 mm diameter imipenem (30 μg/disc, Oxoid UK) was used as a positive control for antibacterial activity. The plates were incubated at 37 °C for 48 h. After incubation, clear area around the wells indicated the inhibition zones, which were measured in millimeters by caliper in order to evaluate the degree of susceptibility of the test organisms and labeled ‘sensitive’ or ‘resistant’ was compared to the standard antibiotics. All experiments were done in a duplicate manner.

2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the agents

MIC was employed to determine minimum concentration of honey which will inhibit growth of the isolated microorganisms. The MIC was carried out using the Mueller-Hinton broth dilution method in serial dilution preparations [13]. A dilution schedule of MIC, growth visibility and non-growth tubes were registered then proceeding with the MBC test [14].

MBC was measured from the broth dilution tests using decreasing concentrations of honey and simulated honey solution by sub-culturing to antibiotic-free Mueller-Hinton agar from tubes showing no visible growth and also from the two dilution suspensions preceding the MIC dilution. A standard inoculum of the microorganism was added to an equal volume of each concentration. Mueller-Hinton broth tubes were prepared once with the crude honey and a second time with simulated honey. All Mueller-Hinton broth tubes were incubated at 35 °C for 24 h. The experiment was done in a duplicate manner for each of the five microorganisms. Results were registered to compare with the media control Mueller-Hinton broth tubes prepared as follows; one broth tube containing the test bacteria, the second broth tube containing the standard antibiotics and the third broth tube containing sterile distilled water. The dilution of product that produced no growth was recorded as the MBC.

3. Results

A total of 90 samples were collected from sputum (n = 55) and throat swab (n = 35). The isolated bacterial species were identified and confirmed as: 26 of *K. pneumoniae* (28.9%), 19 of