Available online on 15.04.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

The Effect of Honey on Bacteria Isolated from Urinary Tract Infections among Patients Attending Ruhengeri Referral Hospital

Ishimwe Alain Prudence [1,2,3]*, Munezero Pierre Celestin [1,2], Migabo Hiberte [1], Itangimbabazi Josue [1], Saramba Eric [2]

[1] *Ines-Ruhengeri, Faculty of Applied Fundamental Sciences, Department of Biomedical Laboratory Sciences, Rwanda*

[2] *University of Rwanda, College of Medicine and Health Sciences, Department of Biomedical Laboratory Sciences, Rwanda*

[3] *Kibogora Polytechnic, Department of Biomedical Laboratory Sciences, Rwanda*

Article Info:

Article History:

Received 19 May 2023
Reviewed 02 July 2023
Accepted 14 August 2023
Published 15 April 2024

Cite this article as:

Alain Prudence I, Pierre Celestin M, Hiberte M, Josue I, Eric S, The Effect of Honey on Bacteria Isolated from Urinary Tract Infections among Patients Attending Ruhengeri Referral Hospital, Journal of Drug Delivery and Therapeutics. 2024; 14(4):10-13

DOI: http://dx.doi.org/10.22270/jddt.v14i4.6104

*Address for Correspondence:

Ishimwe Alain Prudence Ines-Ruhengeri, Faculty of Applied Fundamental Sciences, Department of Biomedical Laboratory Sciences, Rwanda

Abstract

Background: Urinary tract infections (UTIs) occur when bacteria enter the urethra from the skin or rectum and infect the bladder, causing symptoms. UTIs are becoming increasingly multidrug-resistant, with some drugs no longer effective in treatment. According to the National Center for Biotechnology Information (NCBI), honey has been shown to have high osmotic pressure and high acidity that prevent the multiplication of bacteria. This study aimed to evaluate the effect of honey on bacteria isolated from urine samples collected from patients with UTI's attending Ruhengeri Referral Hospital.

Methodology: Urine samples were collected in sterile containers at Ruhengeri Referral Hospital and transported to the microbiology laboratory at the Ruhengeri Institute of Higher Education (INES Ruhengeri). Two types of honey, raw and processed were used as antimicrobial agents on bacteria isolated from UTIs. Various methods were used to isolate and identify bacterial isolates, including culture on solid medium, gram staining, and biochemical tests.

Results: The most commonly isolated bacterium was *S. aureus* (33.3%) which is in the second group of bacteria susceptible to honey, followed by *P. aeruginosa* (13.8%) and CoNS (13.8%). *E. coli*, *K. pneumonia*, and *Streptococcus* spp. each accounted for 11.2%. The former and the latter were the most susceptible bacteria to natural honey honey (mIZ = 36 ±2.83; ±4.24 mm, respectively), while *E. aerogenes* was the least isolated bacterium at 5.5% and the least susceptible to honey.

Conclusion: Honey was showed to exhibit an antimicrobial effect against all bacteria isolated from the UTI, suggesting natural honey as a remedy in controlling urinary tract bacterial infection. Further studies are needed to evaluate its capacity compared to classical antibiotics currently used to treat bacteria in UTIs. The practical way to use honey to get targeted area is also an interested point that need to be investigated.

Keywords: Urinary tract infection; honey; bacteria.

INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections affecting millions of individuals worldwide, with women being particularly susceptible (Bhargava et al., 2022). In most cases, UTIs are caused by coliform bacteria, such as Escherichia coli, leading to symptoms including dysuria, frequency, and lower abdominal pain (Mandracchia et al., 2016). Although antibiotics represent the standard UTI treatment, the rising prevalence of antibiotic-resistant bacteria necessitates the exploration of alternative therapies, including natural products like honey [1].

Honey has been employed for medicinal purposes for millennia and is recognized for its antimicrobial, anti-inflammatory, and antioxidant properties [2]. Recent research has demonstrated the ability of honey to effectively inhibit the growth of various pathogenic bacteria, including those implicated in UTIs [3]. The antimicrobial mechanism of action is of honey is believed to be multifactorial, with elements such as high sugar content, acidity, and hydrogen peroxide production contributing to its antibacterial properties [4]. Despite honey's potential therapeutic benefits, data on its effectiveness in treating UTIs remains scarce, particularly in resource-limited settings. In Rwanda, UTIs are a prevalent especially among women, and are associated with poor hygiene practices and limited access to clean water [5]. A review of UTIs in Rwanda identified E. coli as the most common pathogen and reported high resistance rates to commonly used antibiotics [6]. This underlines the urgent need for alternative therapies to treat UTIs in vulnerable populations.

This study aimed to investigate honey's effect on bacteria isolated from urine obtained from patients suffering from UTIs at the Ruhengeri Referral Hospital in Rwanda.

MATERIALS AND METHODS

Study Setting and Design

This cross-sectional study was conducted at Ruhengeri Referral Hospital in the Northern Province of Rwanda. Upon enrolment,

each participant donated a random urine sample that was transported to the microbiology laboratory at INES Ruhengeri for bacterial culture, identification and sensitivity testing.

Data Collection

The study enrolled consenting patients diagnosed with urinary tract infections who attended Ruhengeri Referral Hospital. A sample size of 30 eligible and consecutive patients who visited the hospital between August 2022 and October 2022 was selected. Inclusion criteria were a UTI diagnosis, while patients with infections at other anatomical sites were excluded. Urine samples were collected in sterile containers and transported to the microbiology laboratory at INES Ruhengeri for isolation and identification of bacteria and susceptibility testing.

Bacterial isolation, identification and susceptibility testing

Each sample was inoculated on different culture media such as Blood Agar, MacConkey and Mannitol Salt Agar using streaking method. After gram staining of each distinct single isolated colony of bacteria, the latter was confirmed using biochemical tests, including catalase, coagulase, citrate test, sugar fermentation and hydrogen sulphide production, motility test, urease test, and indole production.

Antibiotic Susceptibility Test

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method in accordance with the guidelines provided by the Clinical Laboratory Standards Institute (CLSI) on Muller-Hinton Agar (CLSI, 2020). The test organisms were prepared by creating a suspension of 3-5 freshly grown colonies, equivalent to a 0.5 McFarland standard. The swab containing the suspension was swirled to cover the surface of the Muller-Hinton agar. The plates were allowed to dry for 3-5 minutes at room temperature before a sterile dropper was used to apply a drop of honey to the inoculated plates' tip hole. Subsequently, the plates were incubated aerobically at 37°C for 24 hours. The diameter of the clear zone surrounding the drop of honey was measured using a ruler, and the results were reported as the diameter of the zone of inhibition.

Ethical Considerations

The authorization to carry out this study was obtained from both Ruhengeri Referral Hospital Ruhengeri Institute of Higher Education. Each patient has signed the consent form prior to be enrolled into the study. All patients a unique study identifier.

RESULTS

Frequency of bacterial isolates

The most frequently isolated bacteria were *S. aureus* (33.3%), followed by *P. aeruginosa* (13.8%) and Coagulase negative Staphylococcus (CoNS) (13.8%), *E. coli*, *K. pneumonia*, and Streptococcus spp. each accounted for 11.2%, and the least isolated was *E. aerogenes* at 5.5% (Fig. 1).

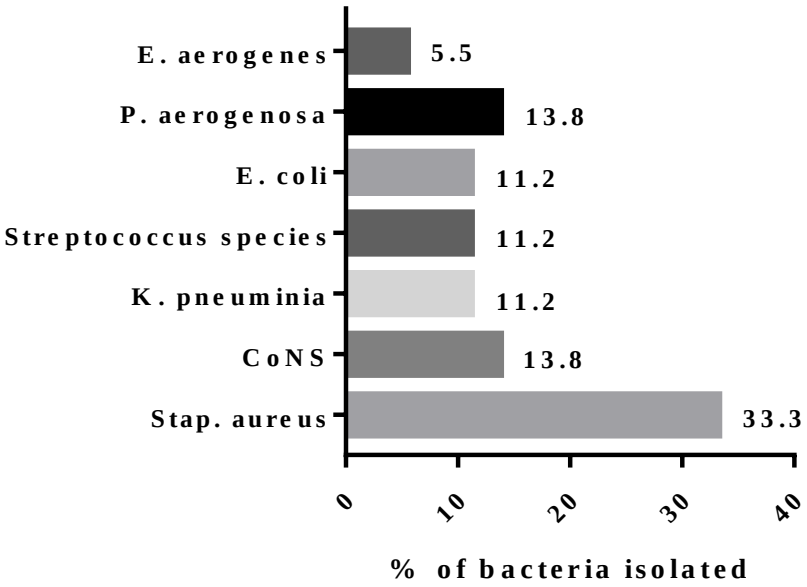

Figure 1: The distribution of bacterial species isolated from study participants

Antimicrobial Effect of Honey

Two types of honey, processed honey and raw honey with no additives or dilution, were evaluated for antimicrobial activity against the bacteria isolated from the study participants. The processed honey did not exhibit significant activity against the isolated bacteria, as evidenced by the absence of any inhibition zone and bacteria growth on the Mueller-Hinton agar (MHA). On the other hand, raw honey demonstrated antimicrobial activity against the bacterial isolates, with varying degrees of inhibition zones depending on the type of bacteria. E. coli and Streptococcus species were the most susceptible to the honey, demonstrated by mean diameter of inhibition zone (mIZ) (mIZ = 36 ±2.83; ±4.24 mm, respectively), followed by *S. aureus* and CoNS (mIZ = 36 ±4.24; ±5.66 mm, respectively), *K. pneumonia* (mIZ = 28 ±5.66 mm), *P. aeruginosa* (mIZ = 26 ±1.41 mm), and *E. aerogenes* (mIZ =24 ±2.83 mm) (Table 1)

Table 1: Mean zone of bacterial growth inhibition by raw honey on different isolated bacteria.

Bacteria	n	Inhibition zone (mm)	Std Dev
E.coli	2	36	±2.83
S. aureus	2	31	±4.24
K. pneuminia	2	28	±5.66
P. aeruginosa	2	26	±1.41
E. aerogenes	2	24	±2.83
CoNS	2	31	±5.66
Streptococcus species	2	36	±4.24

DISCUSSION

Urinary tract infections are caused by a wide range of microorganisms, including bacteria (gram-positive and –negative) and fungi, which are often isolated from patients' urine samples [7](Bhargava et al., 2022). Bacteria commonly associated with UTIs include *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. aerogenes*, CoNS, and *Streptococcus* spp (Hodson & Craig, 2015). Previous studies reported *E. coli* as the most prevalent bacteria causing UTIs [8], typically gaining entry into the urinary tract through stool containing the bacteria [9]. Women are more susceptible to UTIs than men due to their shorter urethra, which is also situated closer to the anus, a source of *E. coli* [9]. K. pneumoniae infections are primarily nosocomial. Individuals with weakened immune systems, such as those who are sick or injured, and those undergoing various medical procedures are at a higher risk of contracting K. pneumoniae infection than the general population [10]. Infections can occur through the use of contaminated medical equipment, such as ventilators and intravenous catheters [11].

In this study Staphylococcus species (S. aureus and CoNS) were most prevalent isolated bacteria and *S. aureus* was more frequent than CoNS. Gessese et al. reported them at the same proportion [12]. Contrary, Flores-Mireles et al. mentioned that CoNS are more prevalent than *S. aureus* [7]. Staphylococcus species are often the predominant bacterial species isolated from patients' urine samples, as it is primarily spread by contaminated hands [12]. Although skin and mucous membranes generally serve as effective barriers against infection, damage to these barriers allows bacteria to penetrate and cause infection [13]. Individuals with weakened immune systems or invasive medical devices, like catheters, are especially vulnerable to *S. aureus* infections [14]. Current results differ from those reported by Loubet et al, that *S. aureus* is an uncommon isolate in urine cultures contributing to only 0.5–6% of positive urine cultures, except in patients with risk factors for urinary tract colonization [1]. This discrepancy might possibly be explained by poor collection of urine specimens leading to contamination [15].

E. aerogenes was the least frequently isolated bacteria from urine samples in the present study, as it is primarily associated with healthcare-related infections and is responsible for numerous nosocomial infections, including UTIs [16]. Enterobacter species can be part of the mammalian gastrointestinal tract's microflora and may be present on human skin surfaces, in water, certain foods, soil, and sewage [17].

The antimicrobial susceptibility test was performed using honey. The properties of honey include; low pH, high osmotic effect, high sugar concentration, hydrogen peroxide generation and a high content of antioxidants, lysozymes, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides, which together possibly contribute to the antimicrobial properties against some bacteria that cause UTIs [18]. The unadulterated quality and composition of honey are essential for it to function as an antimicrobial agent [19].

Artificial or processed honey, prepared using a mixture of mono- and disaccharides at concentrations similar to those found in natural honey, often did not demonstrate effective bacterial inhibition [20]. The osmotic pressure exerted by honey's high sugar content is a crucial aspect of its antibacterial activity, and the level of inhibition depends on honey's concentration [21].

Studies have demonstrated that natural honey can inhibit growth and kill isolated bacteria from UTIs, with varying zones of inhibition [22]. Studies have emphasized on role of hydrogen peroxidase produced by honey in antimicrobial activity [2]. However their mode of action differ depending on the type of the honey used. For instance, manuka honey was used as characteristic of honey with peroxidase activity while pasture honey with non-peroxidase activity [23]. The same authors demonstrated the antimicrobial activity of both honey against CoNS. While in this study the processed honey did not show any effect on isolated bacteria, French et al. demonstrated a weak effect of simulated honey in inhibiting isolates [23].

This study has shown the antimicrobial activity of natural honey to both gram-negative and positive bacteria. In addition to the above-mentioned components of the honey, many other component were mentioned to involve in its capacity to inhibit bacterial growth and these include high viscosity which is mostly due to the high concentration of sugar, and the low content of water. To these, it adds the mild acidity which is an adverse environment for bacterial growth [24]. Hydrogen peroxidase was also described as an important component in antibacterial activity of the honey. However, its activity requires the dilution of the honey to allow the honey-containing glucose oxidase to act on endogenous glucose which in turn produces hydrogen peroxidase [25]. On the other hand, the non-peroxidase antibacterial activity of honey has been documented. One of the components that come in action is the phenolic compound [26]. That is why, manuka honey possesses higher content of phenolic acid than pasture honey [27].

CONCLUSION

With different bacteria that are currently showing resistance to different classical antibiotics, the natural showed to be a remedy to controlling bacteria isolated from the urinary tract. Further studies are of needed to compare the inhibition capacity of natural honey with classical antibiotics. Additionally, in vivo studies will also shed light on the efficacy of natural honey as nutritional regimen. That is why the practical way to use honey to get targeted area is also an interested point that need to be investigated.

ACKNOWLEDGMENTS

We extend our gratitude to Ruhengeri Referral Hospital administration for facilitating this study at their health facilities.

Conflict of interest

Authors declare no conflict of interest

Availability of raw data and material

Raw data and information on material should be obtained from the corresponding author upon request.

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