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Research Article

ANTIBACTERIAL POTENTIAL OF MANUKA HONEY AGAINST EXTENDED SPECTRUM BETA LACTAMASES PRODUCING CLINICAL ISOLATES

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ABSTRACT

Infectious diseases induced by drug-resistant bacteria are one of the major problems in clinical practice. In the ongoing scenario, where the resistant bacteria are spreading widely and limited options for treatment are presently available with antimicrobial agents. The study was conducted to determine antibacterial potential of Manuka honey (MH)BV20+ joint at different strength against extended spectrum beta lactamases (esbl) producing bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (n = 30 for all isolates). Resistance pattern along with minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were analysed. From thirty clinical isolates each of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, no one showed resistance against manuka honey even at 4000 µL/mL dilution but twelve samples of *Escherichia coli* and ten samples of *Klebsiella pneumonia* showed resistance against Ciprofloxacin, used as positive control. The average zone of inhibition of Ciprofloxacin against *Escherichia coli* was 24.14 mm, for *Pseudomonas aeruginosa* it was 26.38 mm and 25.14 mm for *Klebsiella pneumoniae* while the average zone of inhibition of undiluted manuka honey against *Escherichia coli* was 29.38 mm, for *Pseudomonas aeruginosa* it was 28.22 mm and 25.21 mm for *Klebsiella pneumoniae*. MIC and MBC were found to be 4000 µL/mL against *Escherichia coli* and *Pseudomonas aeruginosa* while for *Klebsiella pneumonia* it was 5000 µL/mL. Manuka honey BV20+ joint showed an excellent antibacterial potential against resistant strains of extended spectrum beta lactamases bacteria indicating its significance in clinical practice.

Keywords: Manuka honey, resistance pattern, clinical isolates, potential, clinical practice.

INTRODUCTION

The marked emergence of bacterial resistance to antimicrobials is now considered as an alarming condition. During the last decades, antimicrobial resistance (AMR) has steadily been increasing, especially regarding resistance to quinolones, carbapenem and third-generation cephalosporins[1]. AMR indicates the evolutionary mechanisms occurring in microorganism during treatment which is affecting both developed and developing countries. Antimicrobial resistance is not only an alarming and emerging issue but also as increasing problem of remarkable magnitudes[2]. Additional mutations may compensate for fitness of microbes and can enhance the survival of resistant bacteria[3]. Therefore, it is imperious to decrease AMR developmental pattern to such an extent that maintains the effectiveness of available antimicrobials[4]. For the effective management of bacterial infections, accurate bacterial susceptibility determination to current antibiotics is very essential. The honey extracts, with various solvent, showed good antibacterial properties as compared to recommended antibiotics including ciprofloxacin and tetracycline against gram-negative strains of bacteria. Bactericidal action of the solvent extracts of honey samples were observed against *P. aeruginosa* for which even tetracycline was found ineffective. MIC and MBC values of honey solvent extracts were calculated in the range of 0.625-5.000 mg/ml[13].Currently, various honeys have been marketed with standard label of antibacterial activity. Among them best known is Manuka honey of New Zealand which is produced from *Leptospermum scoparium*[5]. Research has been conducted on Manuka honey of *Leptospermum scoparium* origin which reflected effectiveness of the honey against numerous human pathogens including *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter aerogenes*[6, 7]. Manuka honey, as an anti-bacterial agent, has the ability to treat a variety of illness. It is believed that honey have a broad spectrum of antimicrobial action, different honey, for example, Manuka (New Zealand), Heather (United Kingdom) and Khadikraft (India) differ significantly in activity and antibacterial spectrum[8]. Honey is anti-bacterial and have antibacterial activity equivalent to commercially available Manuka Honey (MH) against bacterial pathogens including both gram-negative and gram-positive[9].

MATERIAL AND METHODS

Collection of clinical isolates

Clinical isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* (30 each) were collected from Civil Hospital Karachi, Pakistan. These pathogens were isolated from urine, blood and pus samples.

Isolation and identification of organism

Isolation of clinical isolates was done on the basis of morphological, culture and biochemical reactions at Civil Hospital Karachi, Pakistan.

Collection of honey sample

Manuka Honey BV20+ Joint was imported from United States of America via Calcomp Nutrition Inc. having voucher no. MHWGBV250-1.

Preparation of honey dilutions

Honey dilutions were prepared immediately prior to testing by diluting honey with distilled water to the required concentration of 2000 µL/ml, 4000 µL/ml, 6000 µL/ml and 8000 µL/ml (v/v). All the samples were incubated for 30 min at 37°C in shaking water bath for solution aeration. Incubation was performed in the absence of light as both glucose and H_2O_2 are sensitive to light[10].

Collection of Antibiotic

Ciprofloxacin (Quinoflox 100 mg/50 mL, Bosch species) was purchased.

Susceptibility testing

Following steps were taken to check the sensitivity and resistance pattern of microorganisms[11].

Preparation of inoculum, broth and media plates

Muller-Hinton medium (Oxoid Ltd; Basingstoke, Hampshire, England) was used to test sensitivity and resistance pattern on clinical isolates. Those colonies of ESBL having same morphological type were selected from an agar plate. Using National Committee for Clinical Laboratory Standards (NCCLS) guidelines, Mueller-Hinton broth and agar medium was prepared. By the use of a sterile wire loop, surface of each colony was touched and then transferred to a tube containing 4 ml to 5 ml of a suitable broth. Broth was incubated at 37°C for 8-24 hours. Bacterial culture suspension having an appropriate turbidity was prepared using 0.5 McFarland standards (McS) as a reference to ensure the number of organisms will be within a given range[12]. In the bacterial suspension, a sterile cotton swab was dipped and then streaked in three directions over the Mueller-Hinton agar surface to obtain uniformity in growth. Plates were dried for ten minutes.

Application of material in well

By using sterile cork borer, wells were made in media with a diameter of 6 to 8 mm and applied 1 ml of Manuka honey undiluted and dilutions of 2000 µL/ ml ,4000 µL/ ml, 6000 µL/ml and 8000 µL/ml (v/v) in punched wells with the help of 3 mL sterile syringe under aseptic conditions. Ciprofloxacin 5 µg/mL was used as positive control and ethyl acetate as negative control[13]. Wells were completely filled to ensure contact with agar. The wells were bored in such a manner that they were not less than 25 mm from each other and were 15 mm from the edge of the plate.

Incubation of plates

After that plates were incubated at 37°C for 24 hours. The diameter of the zones of growth inhibition around each well was measured in mm by using Vernier caliper.

Minimum inhibitory concentration (MIC)

For MIC determination, micro-broth dilution technique was used. Manuka honey dilutions were prepared in distilled water to get concentrations of 1000 µL/ ml, 2000 µL/ ml, 3000 µL/ml, 4000 µL/ml and 5000 µL/ml. 2 ml each of Mueller–Hinton broth and honey were mixed. Precisely, to each of the test tubes, 1 ml of standardized inoculums having 3.3 x 10^6 CFU/ml was added and incubated for 24 hours at temperature 35°C in aerobic condition. Broth and honey containing tubes lacking inoculums served as positive control while as negative control, tubes containing broth and inoculums were used. To determine minimum inhibitory concentration, the tubes were analyzed after incubation period of 24 hours. The lowest concentration showed MIC with evidence of lacking of growth[14, 15].

Minimum Bactericidal Concentration (MBC)

To determine MBC, Mueller-Hinton agar sterile plates were inoculated separately with test tubes that showed absence of growth. The test plates were again incubated for 24 hours at 35°C in incubator and then analyzed. The highest dilution lacking bacterial growth was considered as MBC[14,15].

Table 1: Antibacterial activity of Manuka honey against ESBL producing bacterial strains

Bacterial strains	Test					Control	
	Average diameter of clear zone of Inhibition (mm)						
	Honey mL	Honey dilutions / mL				Ethyl acetate -ve	Ciprofloxacin +ve
	Undiluted	2000 µL	4000 µL	6000 µL	8000 µL	mL	mL
Escherichia coli	29.38	14.45	23.37	25.21	26.87	NZ	24.14
Pseudomonas aeruginosa	28.22	13.44	22.98	24.04	25.77	NZ	26.38
Klebsiella pneumoniae	25.21	12.88	22.11	23.89	24.07	NZ	25.14

(NZ; No Zone of inhibition)

Table 2: MIC and MBC of different dilutions of Manuka honey

Bacterial Strains	MIC					MBC				
	1000 µL	2000 µL	3000 µL	4000 µL	5000 µL	1000 µL	2000 µL	3000 µL	4000 µL	5000 µL
Escherichia coli	D	D	SD	ND	ND	D	D	SD	ND	ND
Pseudomonas aeruginosa	D	D	SD	ND	ND	D	D	D	ND	ND
Klebsiella pneumoniae	D	D	D	SD	ND	D	D	D	SD	ND

Note (MIC; Minimum Inhibitory Concentration, MBC; Minimum Bactericidal Concentration, ND; Not Detected, D; Detected, SD; Slightly Detected)

RESULT

Manuka honey was found to be highly effective against all tested ESBL clinical isolates including *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* even at 4000 µL/mL dilution. The values of zone of inhibition are given in Table 1. MIC and MBC were found to be 4000 µL/mL against Escherichia coli and Pseudomonas aeruginosa while for Klebsiella pneumonia it was 5000 µL/mL. The MIC and MBC values are given in Table 2.

DISCUSSION

The object of this study was to evaluate antibacterial activity of manuka honey against extended spectrum beta lactamases producing bacterial strains. Various researchers have shown that honey exerts antimicrobial activities against various microorganisms[5]. Clinically isolated samples of tested ESBL were evaluated against manuka honey at the different concentration/ dilutions. Manuka honey showed better zones of inhibition even at dilutions as comparable to that of Ciprofloxacin, used as positive control and in some cases more that it. *Escherichia coli* (seven out of thirty samples) and *Klebsiella pneumonia* (ten out of thirty samples) showed resistance pattern against Ciprofloxacin while none of the tested bacterial strains were resistant to manuka honey even at 4000 µL/mL dilution. Manuka honey exhibit an excellent antimicrobial activity against numerous bacterial strains[16,17]. However, poor antibacterial activity of manuka honey has also been observed[18]. Not all manuka honey are antibacterial except the New Zealand one[19]. The average zone of inhibition of ciprofloxacin against *Escherichia coli* was 24.14 mm, for *Pseudomonas aeruginosa* it was 26.38 mm and 25.14 mm for *Klebsiella pneumoniae* while the average zone of inhibition of manuka honey against *Escherichia coli* was found to be 29.38 mm, for *Pseudomonas aeruginosa* it was 28.22 mm and 25.21 mm for *Klebsiella pneumoniae.* Manuka honey at 4000 µL/mL dilution, was also active and zone of inhibition obtained against *Escherichia coli* was 23.37 mm, for *Pseudomonas aeruginosa* it was 22.98 mm and 22.11 mm against *Klebsiella pneumonia* indicating manuka honey is effective against tested ESBL producing bacteria which was not been observed in previous studies[20]. During current study, manuka honey was not only found to be more active against ESBL producing *Escherichia coli* but also equivalent antibacterial activity has not been observed previously against it [21-23]. Natural honey (unheated) has shown broad-spectrum antibacterial action against pathogenic strains of bacteria[18]. Honey also exhibit antimicrobial spectrum because of numerous reasons including reduced water activity and less pH, generation of H_2O_2, carbohydrates, proteins or other unidentified substances[24].

CONCLUSION

Manuka honey showed excellent antimicrobial activity so it can be a good candidate against resistant ESBL producing strains of bacteria. Further research is required to evaluate manuka honey usage as complementary and alternative medicine and / or as an empirical therapy against infections induced by ESBL producing strains of bacteria.

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