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EFFICACY OF HONEY AGAINST BACTERIAL ISOLATES FROM DIABETIC FOOT ULCERS IN PATIENTS SUFFERING WITH IMMUNOSUPPRESSION – AN in vitro STUDY

Medical Science

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ABSTRACT

Introduction: Diabetic foot ulcers in old age people require special attention considering the co morbidities and immune status. Presently many isolates responsible for such infections are multidrug resistant so long course of drug intake is required for a proper healing. The antibacterial effect of honey in such wounds will help to evolve a novel effective treatment method for non-healing diabetic foot ulcer. **Aims and objectives:** The major objective of the study is to find the effectiveness of different types of honey against bacterial isolates from diabetic foot ulcers in patients suffering with immunosuppression, by in vitro laboratory techniques. **Materials and methods:** The study is done with samples collected from 30 cases of diabetic foot ulcers in old age people with immunosuppression from Wayanad and neighboring districts. Isolation and identification of etiological agents, by different bacteriological techniques and in vitro antibiogram study against commonly used antibiotics are carried out. Antibacterial effect of different types of honey available in Wayanad district, at different concentrations, are assessed by in vitro techniques, and minimum inhibitory concentration (MIC) of honey is estimated. **Result:** The study shows that concentrated honey is an effective inhibitory agent for many drug resistant bacteria, but the effect is diminishing on further dilutions Antibiotics are more effective when compared to diluted honey in inhibiting bacterial growth This study showed effectiveness of honey to inhibit the growth of MRSA **Conclusion:** The findings of this study will explore the possibility of honey based drops, ointments and bandages for wound infections. This study will also help to generate data on bacterial etiology of diabetic foot ulcers in geriatrics, especially those which are non-responsive to anti-bacterial, and their antibiogram pattern against commonly used antibiotics.

KEYWORDS

Antibacterial effect, Honey, diabetic ulcers, antibiotic resistance, Immunosuppression.

Introduction:
The management of diabetic foot ulcers in old age people requires an individualises approach considering the patients co- morbidities and functional status. Presently there are isolates with multiple drug resistance which is making wound healing difficult and many a times long course of drugs have to be taken for a healing. This is because of developing resistance by the causative organisms on its inadvertent use. Honey is natural in origin, well known for centuries for its valued place in traditional human medicine, with excellent antibacterial activity [1&2]. It is known to be safe and useful in treating difficult-to-heal infected wounds as well[3].

Wayanad district is very rich in flora and fauna, with a rich natural resource of honey. The tribal people of this district use this natural product as a traditional medicine and also depend on this natural product as a source of income. The results of this study will help to evolve a novel effective treatment for non-healing diabetic foot ulcers using honey - a natural, economic and safer medicine. The findings of this study will explore the possibility of honey based drops, ointments and bandages for wound infections. This study will also help to generate data on bacterial etiology of diabetic foot ulcers in geriatrics, especially those which are non-responsive to anti-bacterial, and their antibiogram pattern against commonly used antibiotics. There is no previous report of such a study conducted in Kerala.

Aims and objectives:
To identify the bacterial agents involved in diabetic foot ulcers in patients suffering from immunosuppression in Wayanad and neighboring districts

To identify the specific bacterial agents involved in chronic, recurrent diabetic foot, non-responsive to antibacterial therapy

To find out the in vitro antibacterial sensitivity pattern of the commonly used topical antibiotics against these bacterial isolates
To find out the efficacy of different types of honey against these isolates at different concentrations by in-vitro methods to find out the minimal inhibitory concentration (MIC) of honey

Methodology
Study materials: A total of 30 Pus samples collected from patients suffering with diabetic non healing wounds. Samples were collected after getting informed consent after educating them about the importance of the study

Centre of the study: The study was conducted in the clinical microbiology Lab under Department of Microbiology, DM WIMS medical college, Wayanad

Duration of study: The study was started on February 2020, Procurement of reagents and chemicals were delayed due to Covid pandemic. The actual bench work started on September 2020 and completed at November 2021

Sample collection: The pus samples and discharges were collected as per standard operation procedure manual (SOPM) and the samples were stored in the refrigerator after proper labelling and entering the details in the register. Pus samples in swabs, bottles and syringes were the medium of collection. The sample ID was the identification tool of the sample throughout the processing periods

Testing methods: Samples were processed without much delay, a maximum of 12 hrs was the scheduled storage time as per the SOPM

Isolation of Bacterial Pathogen: The samples collected were Inoculated in Solid plating media like Blood agar and Mac conkey agar, Gram stained smear was made from all the samples and after inoculating into plating media, enrichment was done for the samples using Thioglycollate medium. The plates and the thioglycollate medium were incubated overnight at 37 degree in an incubator and gram smear was observed under oil immersion objective and observation were recorded

Identification: samples inoculated and kept for incubation were taken out from the incubator and were checked for bacterial growth and the colonies were identified using standard microbiological techniques. The growth were cross related with the gram staining report. The Isolated colonies were kept in slants and stocked in the fridge for studying the MIC testing using honey

Antibiotic susceptibility testing: Antibiotic Susceptibility testing was performed by Kirby–Bauer disk diffusion technique as per Clinical Laboratory Standard Institute (CLSI), 2016 guidelines. The antibiotics tested were selected based on the availability and prescription frequency of these drugs in the study area. (CLSI 2016)[4]

MIC Detection by Honey:

The different types of Honey were collected in sterile screwed caped culture bottle with proper labeling of the type and date of collection. Each honey sample was first filtered with a sterile mesh/gauze to remove debris and then streaked on blood agar plate to check sterility and stored at 2–8°C until used [4].

Minimum bactericidal concentration was tested using standard plate dilution method, wells were made 6 mm diameter, 4 mm deep, and about 2 cm apart, in the agar medium. Using a micropipette, 50 µL of honey with the concentration of 100%, 75%, 50%, and 25% was added to the wells in the plate. The plates were incubated at 37°C for 24 h. The mean diameters of inhibition zones were measured in mm, and the results were recorded.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the antimicrobial agents were determined for each isolate by the tube dilution method[5]. Briefly, ten sterile test tubes were placed in the rack, labeled each 1 through 8. Honey control tubes (HC), broth control tube (BC), and growth control tube (GC) were used as quality controls. One milliliter of freshly prepared nutrient broth was added to each tube, sterilized, and cooled. Then one milliliter of undiluted honey solution 100% was added to test tube number 1 and HC with a sterile micropipette and tips. Then, twofold serial dilution was performed by transferring 1 ml undiluted honey into the second tube with separate sterile micropipette and tips and vortexed for homogenization. After a through mixing, 1 ml was transferred with another sterile micropipette from tube 2 and tube 3. These procedures continued until the eighth tube with a dilution of 1 : 128 was reached, and finally 1 ml was taken and discarded from tube 8. The GC tube that received no honey and BC that received no bacterial inoculums served as growth control while the HC tube that received no bacterial inoculums served as a honey control. Except for the HC tube, each tube was inoculated with 1 ml of the culture of the respective prepared organism. The whole procedures were repeated for all the organisms tested to each of the honey. Tubes were then incubated at 37°C for 24 h and observed by visual inspections for the presence and absence of growth (turbidity). MIC was recorded as the lowest concentration of honey that inhibited bacterial growth (no visible growth or turbidity) [7]

Result:

A total of 30 pus samples were processed and obtained 21 isolates (Table 1). Pseudomonas was the predominant isolate followed by staphylococcus, Escherichia coli, Acinetobacter and one each of Staphylococcus albus, Klebsiella, Enterococci, Barholderia, proteus, citrobacter and Enterobacter spp.

A total of 14 drug resistant isolates were identified from the samples (Table 2), by routine antibiotic sensitivity testing and these isolates were tested with honey. The isolates were ESBL, MDR and MRSA (Table 3) The data obtained showed inhibitory effects of honey at different concentration on the various investigated microorganisms (Table 4).

In all cases of micro-organisms tested, the neat concentration of honey produced a greater inhibition in bacterial growth of Enterobacter but other bacteria, except for E. coli, Pseudomonas and Acinetobacter showed greater susceptibility to the antibiotics and greater inhibition than honey. The data on MIC suggest that the most susceptible micro-organism to honey is S aureus >Klebsiella= E. coli= Pseudomonas. The bactericidal effect was mostly reported in undiluted honey and it has inhibited almost all resistant strains

Bacterial Isolates from wound swabs (Table No 1)

No	Name of the organism	Number of isolates
1	Pseudomonas	5
2	Staphylococcus aureus	4
3	Escherichia coli	3
4	Acinetobacter	2
5	Staphylococcus albus	1
6	Klebsiella	1
7	Enterococci	1
8	Burholderia	1
9	Proteus	1
10	Citrobacter	1
11	Enterobacter	1
	Total	21

Drug resistant isolates (Table 2)

No	Drug resistant isolates	Number of isolates
1	Pseudomonas	4
2	Staphylococcus aureus	4
3	Escherichia coli	3
4	Enterobacter	1
5	Klebsiella	1
6	Acinetobacter	1
	total	14

Details of drug resistance shown by the isolates (Table 3)

No	Isolate	ESBL	MDR	MRSA
	Pseudomonas	4		
	Staphylococcus			4
	Escherichia coli	3		
	Enterobacter	1		
	Klebsiella		1	
	Acinetobacter		1	
	Total (14)	8	2	4

Wound infection and associated characteristics of the patients

GENDER	TOTAL CASES	NO OF ACTUAL INFECTIONS IDENTIFIED	DRUG RESISSTANT STRAIN
MALE	18	14	8
FEMALE	12	7	6

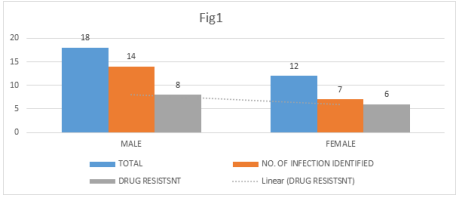


Figure 2

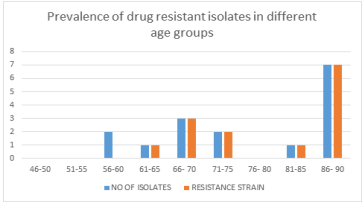
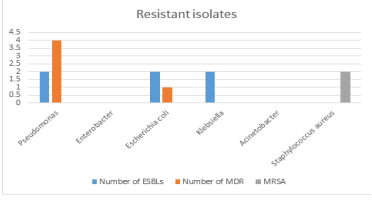


Figure 3



MBC (%) of honey against resistant strains (Table 4)

Organisms	MIC (Mg/ml)	MBC (mg/ml)
Pseudomonas	undetermined	Undetermined
E.coli	128	Undiluted 100 %
Klebsiella	128	Undiluted 100%
Enterobacter	128	128
Acinetobacter	undetermined	Undetermined
Staphylococcus aureus	32	64

Salient Research Achievements:

1. Concentrated honey is an effective inhibitory agent for many drug resistant bacteria, but the effect is diminishing on further dilutions
2. Antibiotics are more effective when compared to diluted honey in inhibiting bacterial growth
3. This study showed effectiveness of honey to inhibit MRSA

Conclusion

Wound infections have been a problem in the field of medicine for a long time, and the problem complicated more recently because of

increased antimicrobial resistance. This is a problem too for public, researchers, clinicians, and drug companies looking for effective drugs. Therefore, antimicrobial resistance may increase complications and costs associated with procedures and treatment that leads to a continued search for new agents.

Honey can be definitely used as a treatment method for healing wounds in patients having drug allergy, Immunosuppression and to prevent MDR bacterial colonization.

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