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Effect of turmeric as antimicrobial agent in MDR uropathogen in diabetic and non-diabetic patients in Nashik

Jayashree Pagar, Hamde Venkat
Department of Microbiology, Yogeshwari Mahavidyalaya, Ambajogai, Maharashtra, India

Abstract
Diabetes is a group of metabolic disorders characterised by high blood sugar levels over a prolonged period. UTI (Urinary Tract Infections) among Diabetic patients is common. The escalating anti-microbial resistance cause recurrent UTI. In recent years Multi Drug resistance by pathogenic bacteria is recorded all over the world. Many plants and their products with pharmacological and biological activities can be used to treat these MDR pathogens. Turmeric one of the important foodstuff all over the world is also used as herbal medicines in several diseases.
In this study 112 diabetic and 188 non-diabetic patients samples with symptoms of UTI were collected and their and urine samples were processed according to Clinical Laboratory Standard Institute (CLSI) guideline. Out of which 23 isolates were found to be MDR. (18.4 %)
The extracts of Turmeric in various solvents like Ethanol, Methanol, acetone were prepared and the sensitivity of the MDR uropathogen were carried out with the tuemeric extracts. The results were noted down and the effect of turmeric as antimicrobial agent on these Multi Drug Resistant pathogen was studied.

Keywords: uropathogen, multi drug resistant

Introduction
Urinary tract infections (UTIs) are some of the most common infections in human worldwide, this is second most common type of infection and occurring from neonate to all age group (Raju and Tiwari 2004). Nearly 10 % of people suffer from UTI during their life time (Delanghe *et al*, 2009: Hoberman and Wald, 1997). It is estimated that 150 million UTIs occur yearly on a global basis.
Urinary Tract infections are of two types depending upon parts of urinary system they affect

1. Cystitis (Lower Urinary Tract infections including infection of Bladder, Tubes carrying urine from bladder to Vagina or penis.)
2. Pyelonephritis (Upper Urinary Tract infections including infection of kidneys and Ureter) [6] In patient over 65 years the prevalence of UTI is observed in both the sexes (Geerling *et al*., 2000).

Diabetes Mellitus is a group of metabolic disorder characterized by high blood sugar level a prolonged period. As of 2017 as estimated 425 million people had diabetes worldwide. This represent 8.8 % of the adult population with equal men and women. Presence of sugar in urine promotes bacterial growth [3, 4], lazy bladder that does not empty completely due to autonomic neuropathy [5, 6], Various impairment in in an immune system [1, 2] all these factors contribute to UTI in diabetic patients. As per 73 rd Scientific Session of American Diabetic Association report 9.4 % of people diagnosed with diabetes 2 had a UTI compared to 5.7 of non-diabetic people. The antimicrobial treatment given to the patient before report of urine culture tends to resistance towards the antibiotics (Tambekar and Dhanorkar, 2005). Due to increasing number of resistant strain of bacteria to various antibiotics their effective ness is decreasing. Medicines derived from plants have played an important role in our health care. The pharmacological and biological activity of the natural products derived from the plants are used in drug discovery, drug design [7]. In Ayurveda plant based drugs or formulations are suggested for so many diseases, infections even cancer. 877 drugs were introduced between 1981 and 2002 61 % were traced. (Newman and Cragg 2007).
Turmeric is widely used as a spice, Due to its colour and flavour used widely. In manufacturing various food products like dairy product, bakery products, popcorn, cereals, sweets, yogurts, gelatin, commercial curry powder etc. It is a product of Curcuma longa. It is a rhizomatous herbaceous plant and belongs to ginger family Zingiberaceae. It has more than 100 components like Turmerone- a volatile oil, curcuminoid – colouring agent. Curcuminoid consists of natural antioxidants. (Rubey *et al* 1995, Selvam *et al* 1995). Nutritional assays have shown that 100 gm of turmeric contain 390 Kcal, 10 g total fat, 3 g saturated fat, 0.2 g Calcium, 10 mg Sodium, 2500 mg Potassium, 0.26 g Phosphorous, 47.5 mg iron, 0.9 mg Thamine, 0.19 mg Riboflavin, 4.8 mg Niacin, 50 mg ascorbic acid, 69.9 g carbohydrate, 3 g sugars, 8 mg protein, 21 g dietary fiber (Balkrishnan 2007). Turmeric is also good sourse of Omega 3 fatty acids and alpha linolenic acid 2.5 %. (Gaud, Polasa and Krishnaswammy 1993). The aim of the study is to identify and isolate Multi Drug resistant Uropathogen among diabetic and non-diabetic patients in Nashik and compare their sensitivity with the herbal extract.

Material and method
The study was done in Nashik Speciality Laboratory Pvt. Ltd. In this study we selected total 300 patients from Nashik, 112 diabetic and 188 non-diabetic patients with symptoms of UTI

and collected their Midstream urine samples. All samples received in the laboratory guidelines were processed according to Clinical Laboratory Standard Institute (CLSI). Antimicrobial susceptibility Pattern was determined by disc diffusion method. The turmeric powder and raw turmeric tuber was collected. The turmeric tuber was disinfected with 0.5 % Mercuric chloride solution, washed with strrrile distilled water, It was crushed and the juice was extracted. The crude extract was done in various solvents (aqueous, Methanol, Ethanol, Acetone in 1: 10 proportion. Soaked sample were kept in rotary shaker

Overnight maintained at constant speed of 150 rpm in airtight glass bottles. After 24 hrs of soaking, sample were filtered through Whatman no 1 filter paper. Sterile Disc were soaked in the extract and dried. The sensitivity of MDR uropathogen were tested against these disc soaked in turmeric extract along with the negative cotrols (Disc soaked and dried in solvents) By Kirby Bauer Method (Chattapadhyay *et al*., 2007)

The results were noted.

Results and Conclusions

a. Isolation of pathogens from UTI Patient.

Total 112 pathogens isolated from Dibetic patients suffering from Urinary Tract Infection. Morphological, cultural and some biochemical characters were studied and tentatively identified up to genus level. These cultures periodically subcultured on Nutrient Agar slants. Out of which 23 isolates were found to be MDR.

b. Preparation of turmeric extract.

Turmeric oil extract was isolated as described in Funk *et al*. (2010) with some modification. About 1.00 g of dried powdered was mixed 500 ml each of distilled water, Ethanol, methanol, and Acetone in flasks separately and kept at room temperature for 7 days. During this period, shaking of the flasks was performed daily. The solvent soluble compounds were filtered using double filter paper (Whatman™). Fresh solvents were added into the used plant material and the process was repeated three times. The filtered solutions containing plant compounds were dried by rotary evaporator.

c. Antimicrobial activity of turmeric extract by disk diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of turmeric was carried by disc diffusion assay as described in Bauer *et al*. (1966). Briefly, for disc diffusion assay, filter paper discs (Whatman no. 1) of 8 mm diameter were prepared and sterilized. Using sterile forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganisms. Two different concentrations of turmeric extracts/oil (6 and 12 µg in DMSO) were aseptically transferred to these discs. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were measured. The data was used for calculating percent inhibition of growth.

For this study we collected samples from 300 patients in Nashik having symptoms of UTI, including 112 diabetic and 188 non-Diabetic. According to Faisal Abdullah *et al* (2017) UTI are most common infection seen in diabetic patient. Among these 112 diabetic patients, 59 were suffering from UTI (52.67 %). Chaudhary *et al*, (2014) [9] mentioned E. coli to be a most frequent uropathogen In diabetic patients. Also Shill *et al* (2010) also stated E.coli to be the commonest uro-pathogen in diabetic patient followed by Klebsiella and Streptococcus. The pathogens we found are *E coli (57.6%), Enterobacter (3.4 %), Enteroccoccus (6.77%), Klebsiella (11.86%), Staphylococcus (8.47 %), Streptococcus (1.69 %), Candida sp (11.86 %), Morganella morganni (1.7%), Pseudomonas sp (5.08 %) and Citrobacter (1.7 %).*(As Shown in Chart no 1).Among 188 non- diabetic patients 66 patients (35.1 %) were suffering from UTI. The isolated pathogens from these samples were *E coli (40.9 %), Enterobacter (10.6 %), Staphylococcus (7.57 %), Streptococcus sp (3.03 %), Pseudomonas (7.57 %), Klebsiella (13.64 %), Candia (4.54 %) and Enterococcus (10.6 %) sp.* As shown in Chart no 2.The management of UTI is becoming measure problem due to increasing resistance of bacteria to various antibiotics use since decades (Gupt *et al* 2001.)

The response of the Uro-pathogen was noted to different antibiotics. (As shown in Table no 1& Table no 2) Good Sensitivity was observed in 102 uropathogen while 23 isolate were found to be MDR (18.4 %). (As shown in Chat 3)The MDR uropathogen were found to be resistant to all Turmeric extract in Various solvents. (As shown in the figure no 1

Table and Figures

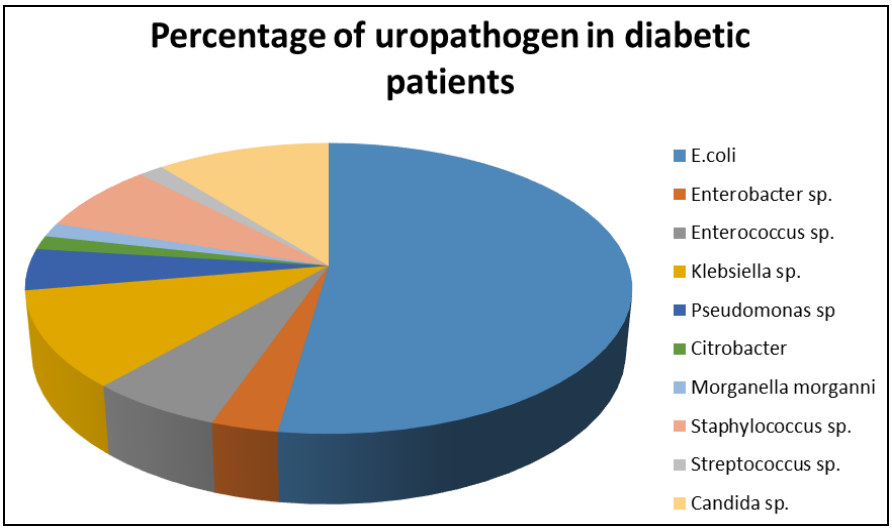

Fig 1: Pie Chart showing the percentage of Uropathogen isolated from Urine of Diabetic patients.

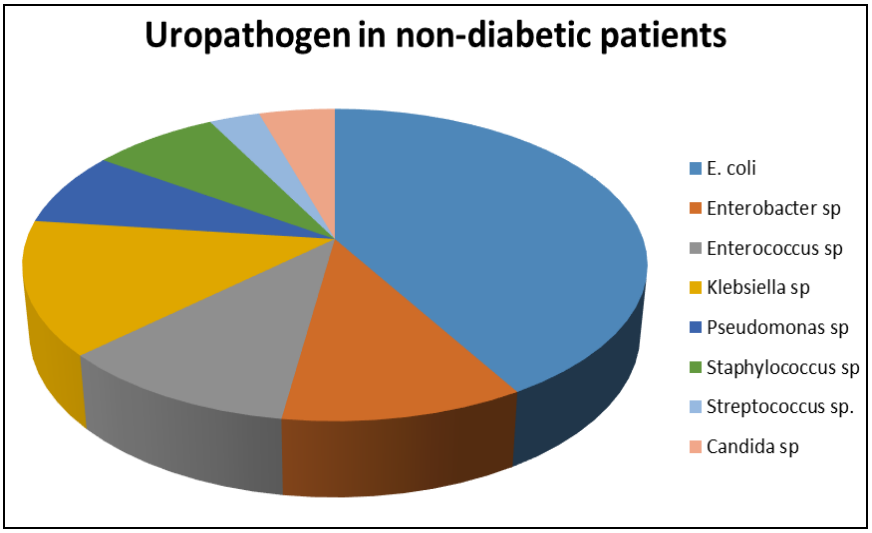

Fig 2: Pie Chart Showing Percentage of Uropathogen isolated from urine of non-diabetic patients.

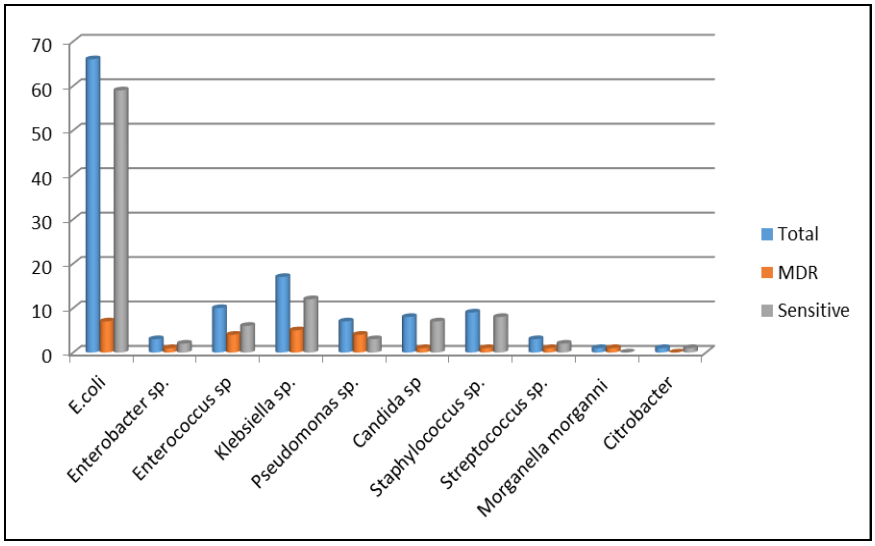

Fig 3: Percentage of MDR.

Table 1: Antibiotic resistance shown by Gram positive Bacteria

Antibiotics	AMK	AMP	AMOX/ CLAV	CEFO	CFZ	CEF	CLIN	COT	LEVO	LENO	MERO	OFL	TEI	NFT	VAN
Isolate															
Ent. c 1	R	S	S	R	R	R	R	R	S	R	S	R	S	S	S
Ent. c 2	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R
Ent. c 3	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S
Ent. c 4	R	R	R	R	R	R	R	R	S	R	R	R	S	I	S
Strepto	R	R	S	I	R	R	R	R	R	R	R	R	R	S	R
Staph.1	R	R	S	S	R	R	R	R	R	R	S	R	R	R	R

Where AMK: Amikacin, AMP: Ampicillin, AMOX/CLAV: Amoxycillin/Clavulunic Acid, CEFO: Cefoparazone, CEF: Ceftazidine, CEF: Cefuraxime, CLIN: Clindamycin, COT: Cotrimoxazole, LEVO: Levofloxacin, LENO: Lenozolid, MERO: Meropenem
OFL: Ofloxacin, TEI: Teicoplannin, NFT: Nitrofurantoin, VAN: Vancomycin, R: Resistant, S: Sensitive, Ent. c: *Enterococcus*, Strepto: *Streptococcus*, Staph *: Staphylococcus.*

Table 2: Antibiotis resistant shown by MDR Gram negative bacteria.

Antibiotics	AMK	AMP	AMOX/ CLAV	CEF	CFR	CFT	CFTR	COL	CO-TRI	DOXY	IMI	MERO	NET	NFT	Piper/ Tazo	OFL
Isolate																
Ec 1	R	R	S	R	R	R	R	S	R	R	R	R	R	S	R	R
Ec 2	S	R	R	R	R	R	R	S	R	R	R	R	S	S	R	R

Ec 3	R	R	S	R	R	R	R	S	R	R	R	R	S	S	R	R
Ec 4	R	R	R	S	S	R	R	S	R	R	R	R	R	I	R	R
Ec 5	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R
Ec 6	S	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R
Ec 7	R	R	R	R	R	R	R	S	R	R	R	S	S	S	R	R
Kl 1	R	R	R	R	R	R	S	R	R	R	R	R	S	S	R	R
Kl 2	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Kl 3	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R
Kl 4	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R
Kl 5	S	R	R	R	R	R	S	R	R	I	R	R	S	R	R	R
Ps 1	R	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R
Ps 2	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R
Ps 3	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R
Ps 4	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Entb	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R

Where Ec: E. coli, Kl: Klebsiella, Ps: Pseudomonas, Entb: Enterobacter.

AMK; Amikacin, AMP: Ampicillin, Amox/Clav: Amoxycillin/Clavulunic Acid, CEF: Cefixime, CFT: Ceftazidine, CFTR: Ceftriaxone, COL: Colistin, CO-TRI: Cotrimoxazole, DOXY: Doxycycline, IMI: Imipenem, MERO: Meropenem,

NET: Netilmycin, NFT: Nitrofurantoin, PIPER/TAZO: Piperacillin /Tazobactum, OFL: Ofloxacin, R: Resistant, S: Sensitive.

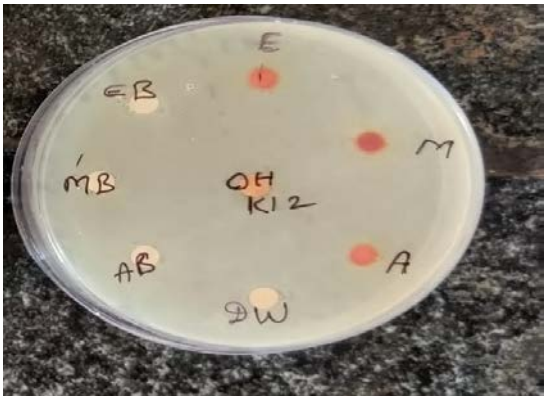

Fig 1: showing resistance of MDR uropathogen to turmeric extract d

Where E: Tuemeric extract in Ethanol, M: Turmeric extract in Methanol, A: Turmeric extract in Acetone, EB: Ethanol blank, MB: Methanol blank, AB: Acetone Blank, DW: Aquous extract of turmeric, OH: Turmeric tuber extract Kl 2: MDR strain of Klebsiella sp.

Conclusion

Our study exhibited a negligible antimicrobial activity of curcumin. The *in vitro* tests included over 23 bacterial species. To our knowledge, the minimum inhibitory concentrations (MICs) of this natural plant compound against planktonic forms of pathogens were determined for the first time. Similarly, the effects of curcumin on Escherichia coli, and Proteus mirabilis had not yet been studied

UTI is in diabetic patient is more 52.67 % while in non-diabetic patient it is 35.1.

E. coli was found to be more predominant uropathogen in diabetic and non-Diabetic patients. (48.8 %).

Among 125 uropathogen 23 were found to be MDR.

The MDR resistant uropathogen also show resistance to the extract of turmeric in

Various solvents. Our research does not end the scientific cycle on evaluation of curcumin activity, but it opens the scientific window to the genetic investigations

Further studies are needed using different food ingredient with medicinal values.

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