SPECTRUM OF UROPATHOGENS OF DIABETICS ALONG WITH ANTIBACTERIAL ACTIVITY OF HERBAL PLANTS AND PROFILE OF ANTIMICROBIAL RESISTANCE

Microbiology

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ABSTRACT

Uropathogens are mainly the pathogens which cause Urinary Tract Infection. They are also among the most common bacterial infections in humans, both in the community and hospital settings occur in all age groups, in both genders, and usually require urgent treatment. A total of 30 urine samples were studied from various pathology laboratories and out of 30 samples 25 samples shows presences of uropathogens. The most frequent isolate was *E. coli* while *S. aureus* was less frequent. Gentamycin, Ciprofloxacin and Erythromycin were most susceptible drugs against uropathogens in patients with diabetics.Antibiotic susceptibility don't differ significantly between Diabetic and Non-diabetic patients.Our study concluded that extract of *C. cassia* especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens. The main objective of the study would be to determine the risk factors, causative organisms and antimicrobial susceptibility of UTI in diabetics and non-diabetics seeking treatment. However, with the advent of modern medicine, the use of select antibacterial agents and antibiotics for the treatment of UTIs was put into practice.

KEYWORDS

Uropathogens, antibacterial activity, antimicrobial activity.

INTRODUCTION:

Uropathogens are mainly the pathogens which cause Urinary Tract Infection. They are also among the most common bacterial infections in humans, both in the community and hospital settings occur in all age groups, in both genders, and usually require urgent treatment. Diabetes mellitus is one of the most challenging health problems of 21st century and is the fifth leading cause of death in developed countries. Diabetes mellitus is a metabolic disorder associated with long term vascular complications leading to morbidity and mortality. The main objective of the study would be to determine the risk factors, causative organisms and antimicrobial susceptibility of UTI in diabetics and non-diabetics seeking treatment. Diabetes causes several abnormalities in the host system that increases the risk of urinary tract infection. The antibiotic resistance which, today, is a serious issue worldwide has invariably been resulted from extensive use of antimicrobial agents. Studies have shown that diabetes by itself does not seem to influence the different uropathogens and their susceptibility patterns to antimicrobials. Many herbs contain dozens of active constituents that combine to give the plant its therapeutic value. The aqueous and ethanol and acetone extracts were studied for their antibacterial effect against strains and results indicated that the extracts were bacteriostatic at higher concentrations. For this purpose the study had been designed to evaluate the spectrum of the uropathogens and their profiles of antimicrobial resistance on a series of diabetic and non-diabetic patients. . The main objective of the study would be to determine the risk factors, causative organisms and antimicrobial susceptibility of UTI in diabetics and non-diabetics seeking treatment. However, with the advent of modern medicine, the use of select antibacterial agents and antibiotics for the treatment of UTIs was put into practice.

MATERIAL AND METHODS:

The urine samples of both diabetic as well as non diabetic patients were collected. The clean catch midstream urine samples were collected in sterile wide mouth container. Samples were collected from different laboratories and tested for presence and identification of various uropathogens. Isolation and identification was done on the basis of cultural, biochemical and enzyme study (Bergey's Manual of Determinative Bacteriology, 1923). Susceptibility to antimicrobial agents was determined by the Disc Diffusion method of Kirby -Bauer on Muller-Hinton agar as described by the Clinical and Laboratory Standard Institute. The Muller Hinton agar plates were prepared for each isolates in sterile condition. Each bacterial isolate was inoculated onto the plate using sterile cotton swab. The cotton swab was dipped into the test suspension and was inoculated evenly onto the agar plates. After spreading the colony on Mueller-Hinton agar, the antibiotics imprignated disks are placed after 15 minutes on the plates by sterile forceps. Then, the plates were incubated at 37°C for overnight (18- 24 hours). A clear zone or ring was observed around the disk after incubation when the agent inhibits growth and the diameter of zone of inhibition across the centre of the disks has been measured in millimeters by using a ruler and the results are interpreted according to the CLSI guidelines in the table. The sensitivity of the isolates to the antibiotics was recorded as susceptible, intermediate and resistant by referring the zone of inhibition size. Plants activity was also checked against various uropathogens obtained. The various parts of the plants were used like bark and the fruit. The plants used to study the activity were *C. cassia (Dalchini)* and *Piper nigrum (Black pepper).* Fine powder was prepared of the dried parts of the plants and dissolved in three extracts i:e acetone, ethanol, and aqueous in varying concentrations that are 0.5%, 1%, 2% and 5%. The medicinal plants activity was checked using well diffusion method, different organic solvents such as Acetone, Ethanol and Aqueous extracts was used for the study. The plates were then incubated into the incubator for 18-24 hrs and observed for the activity. The presence of zone indicates the antibacterial activity of the extract used.
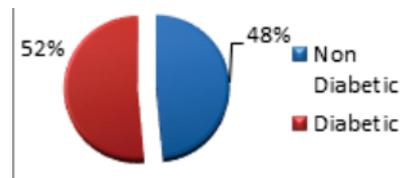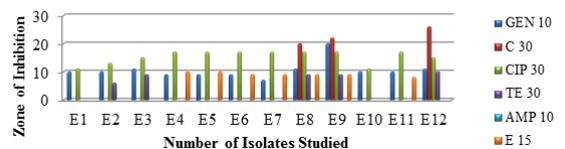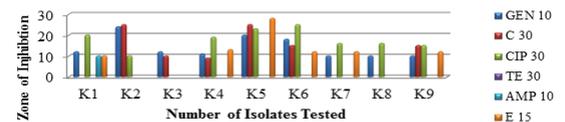
Results :-

Fig: 1 Graph showing distribution of positive UTI cases in Diabetic and Non-diabetic patients.

(E1, E2,…..E12) bacterial isolates studied for Isolate 1.

(K1, K2,…..K9) bacterial isolates studied for Isolate 2.
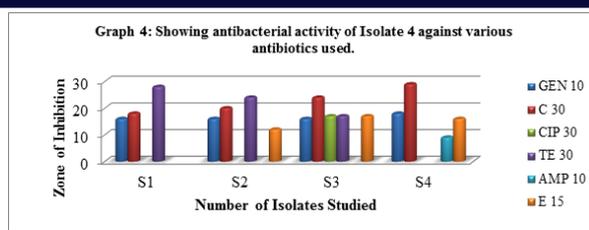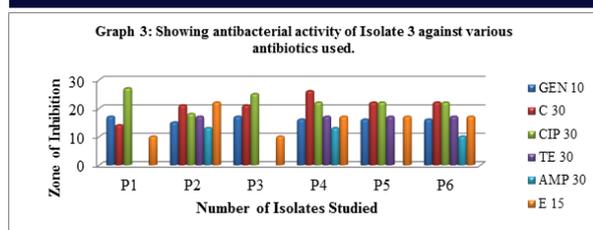
(P1, P2,…..P6) bacterial isolates studied for Isolate 3.   (S1, S2,S3, S4) bacterial isolates studied for Isolate 4.

GEN10: Gentamycin, C30: Chlororamphenicol, CIP30: Ciprofloxin, TE30: Tetracycin, AMP10: Ampicillin, GEN10: Gentamycin

Table 1: Antimicrobial activity of *C.cassia (Dalchini)* against UTI pathogens obtained from Diabetic and Non-diabetic patients.

Plant name and part used	Percentage of solvent used	Zone of inhibition in (mm)											
		Isolate 1			Isolate 2			Isolate 3			Isolate 4		
		A	E	W	A	E	W	A	E	W	A	E	W
C.cassia (bark)	0.5%	10	06	-	-	-	-	07	-	-	14	-	-
	1%	11	12	-	14	-	-	-	13	-	12	13	-
	2%	10	10	10	-	13	-	12	10	-	14	14	-
	5%	14	17	15	15	15	-	-	13	-	17	16	-

A: Acetone, E: Ethanol, W: Aqueous

Table 2: Antimicrobial activity of *P.nigrum (Black pepper)* against UTI pathogens obtained from Diabetic and Non-diabetic patients

Plant name and part used	Percentage of solvent used	Zone of inhibition in (mm)											
		Isolate 1			Isolate 2			Isolate 3			Isolate 4		
		A	E	W	A	E	W	A	E	W	A	E	W
P.nigrum	0.5%	-	-	-	-	-	-	07	-	-	-	-	-
	1%	-	09	-	08	-	-	-		-	-	07	-
	2%	10	10	-	-	08	-	06	07	-	07	08	-
	5%	10	08	-	08	07	-	-	08	-	07	07	-

A: Acetone, E: Ethanol, W: Aqueous

Discussion :-Antibiotic therapy is the first and foremost for UTI in which invasive agents are controlled. Therefore a correlation with the over use of antimicrobial and overuse of antimicrobials and increasing emergence of resistant bacteria seems natural. The knowledge regarding the prevalence of different micro-organisms and antibiotic susceptibility is important for treating physician for proper antibiotic prescription.

A total of 30 urine samples were collected from various pathology laboratories out of 30 samples 25 samples shows presences of uropathogens. The samples of both Diabetic and Non-diabetic patients were collected. Out of 10 samples collected for Non-diabetic patients 8 samples showed positive results out of which 30% were males and 50% were females. Out of the 20 samples studied for Non-diabetic patients 17 were found positive of which 30% are males and 55% females. The overall percentage of the UTI infection studied showed that 52% were Non-diabetic while 48% were Diabetic patients as shown in and Figure: 1

In our study it was found that positive UTI cultures were shown by Diabetic patients then that of the Non-diabetic ones. It was also found that the positive cases showing UTI's were more in females i:e 55% and 50% in Diabetic and Non-diabetic respectively than that of the males which was found to be 30% in both the Diabetic and Non-diabetic cases. This is comparable to the gender wise distribution reported by Muhammad *et al.,* (2014). showed an increased percentage of positive UTI cases i:e upto 53% in 79 bacterial isolates studied and 47% in males in 70 isolates studied. Also the number as reported by Prakash *et al.,* (2014) showed that out of 100 Diabetic and Non-diabetic samples studied 51% were females and 49 % were males.

In our study of 31 isolates most of the bacterial isolates obtained were *E.coli* constituting of about 39% . This is comparable to the result of Prakash *et al.,* (2014). They reported that *E.coli* was most commonly isolated pathogens in UTI accounting about 45% in Diabetic and 63% in Non-diabetic patients. The second most frequently isolated bacterial uropathogens in our study was *Klebsiella spp* showing of about 29% of all the bacterial isolates studied. The third predominating species was found to be *Pseudomonas spp* which was found to be 13% and least isolated species was *S. aureus* which was found to be 13% in all the bacterial isolates studied. These results are comparable to the findings of Fawwad *et al.*, (2014) who reported that *E.coli* (72%) was the most

common uropathogen isolated followed by *Klebsiella spp* (14%), followed by *Acientobacter* (6.9%) and *Proteus vulgaris* (3.90%).

Graphs shows the susceptibility pattern of the various bacterial isolates studied against the antibiotics used. In our studies most of the bacterial isolates studies were sensitive to Gentamycin. The finding was comparable to the results as reported by Muhammad *et al.,* (2014), showing that Gentamycin was sensitive to most of the isolates. It was found that all the *E.coli* isolates studied were found sensitive to Gentamycin. Similarly they also showed that 69.3% *E.coli* bacteria sensitive to Gentamycin and 72.2% *S.aureus* bacteria found sensitive to Gentamycin. In our studies it was found that almost all the bacterial isolates studied were found sensitive to Gentamycin. Similarly in the results reported by Njuda *et al.,* (2013) showed that the most of the bacterial isolated were highly sensitive to Gentamycin (88.6%), Imipenen (87.9%), Nitrofurantoil (79.5%), Amikacin (88.3%).

Similarly in our studies it was found that most of the bacterial isolates were also sensitive to Ciprofloxin. Whereas the findings a reported by Muhammad *et al.,* (2014), was that about 69% were sensitive and 31% were resistant to Ciprofloxin for *E.coli* isolates, and68.305 were sensitive and 31.7% were resistant to ciprofloxin for *S.aureus* isolates studied. According to our results out of 4 bacterial isolates obtained for *S.aureus* 3 isolates were found sensitive to Ciprofloxin and 1 isolate was found to be resistant. These results were found to be contrast in the findings reported by Fawwad *et al.*, (2014) who found that 14.29% of *E.coli* was found to be sensitive while the rest were resistant to Ciprofloxin also none of the *Klebsiella spp* showed sensitivity to Ciprofloxin according to his findings whereas our results output that all the *Klebsiella spp* bacterial isolate studied for Ciprofloxin showed sensitivity to the antibiotic except 1 isolate which found to be resistant as shown in Table 4.

Almost all isolates were found resistant to Amphicillin in our study for *E.coli* bacterial isolates. But comparatively in the study as reported by Acharya, *et al.*, (2015) found that 12.5% bacterial *E.coli* was found sensitive to Amphicillin. Also according to our studies all *Klebsiella spp.* isolates studied were found to be resistant to Amphicillin.

It was reported from our studies that Gentamycin, Chloramphenicol, Ciprofloxin and Erythromycin was sensitive to most of the bacterial isolates studied. Whereas comparatively to the study of Randhir *et al.,* (2016) it was found that Levofloxin, Ofloxacin and Ciprofloxin were most sensitive antibiotics for several uropathogens.

S.aureus isolates in our studies showed sensitivity to Tetracycline which was found to be opposite to that of the findings as reported by Randhir *et al.,* (2016) which was found to be decreased to 38.5% in his study for a study of total 40 *S.aureus* isolates. In all the *E.coli* studied isolates in our study it was shown that the least zone if inhibition was shown to Tetracycline while the highest zone of inhibition was shown by *S.aureus* to Chloramphenicol. As shown in graphs 1,2,3,4.

All the four bacterial isolates obtained were then further tested for activity of plant extracts. The plant extract was dissolved in Aqueous solution as well as organic solvents i:e Acetone and Ethanol and the sensitivity testing was carried out. The plant extracts used was *C.cassia* commonly called *Dalchini.* Among the various percentages of solvents used to study the activity of *E.coli* the highest activity was shown by 5% (17mm) concentration of Ethanol extract. Zone of inhibition was shown by both the organic solvents i.e. Acetone and Ethanol extract while the Aqueous extracts shows inhibition in the 2% (10mm) and 5% (15mm) concentration used. The highest inhibition activity was reported by Ethanol extract which was found to be 17mm followed by Aqueous extract which was 15mm and Acetone extract 14mm for 5% concentration used.

These findings are comparable to the results reported by Sharma *et al.,* (2009) revealed that the Acetone and Ethanol extract exhibited broad spectrum activity against tested isolates as compared to Aqueous extracts.

Similarly in our findings for *Klebsiella spp*, *Pseudomonas spp* and *S.aureus* no activity against Aqueous extracts was seen. This in contrast to the findings as reported by Sharma *et al.,* (2009) which showed as zone of 6mm for *Pseudomonas spp* in the Aqueous extract while the highest activity was reported in the Ethanol extract for *Klebsiella* and *Pseudomonas spp*.

S. aureus isolate was found to show activity against Acetone and Ethanol extract with 17mm and 16mm zone at 5% concentration used while no activity was to be found in Aqueous extract as shown in Fig:5.4

The *P. nigrum* extract used showed negligible activity against the UTI pathogens showing negative results for obtained isolates.

CONCLUSION: The most frequent isolate was *E. coli* while *S. aureus* was less frequent. Gentamycin, Ciprofloxacin and Erythromycin were most susceptible drugs against uropathogens in patients with diabetics.Antibiotic susceptibility don't differ significantly between Diabetic and Non-diabetic patients.Our study concluded that extract of *C. cassia* especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens.

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