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Prevalence of Extended-Spectrum Beta-Lactamase-Producing Pathogens From Urinary Tract Infected Samples and Their Sensitivity Pattern Against *Withania somnifera* L

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Background: Urinary tract infections (UTI) caused by resistant bacteria are becoming more prevalent. The spread of extended-spectrum beta-lactamase (ESBL)-producing bacteria worldwide has become a serious public health issue among healthcare centers and in the community. Drugs and many secondary metabolites from medicinal plants have proved to be economical and effective against many pathogens.

Objectives: The present study was designed to detectext ended-spectrum beta-lactamase (ESBL) producers and their sensitivity pattern against Extracts of *Withania somnifera*.

Patients and Methods: Infected urine clinical samples were collected aseptically and processed immediately for the isolation of pathogens. Isolated pathogens were identified based on physiological characters and their ESBL pattern was determined by the stroke method. The sensitivity of ESBL strains against *Withania somnifera* was evaluated by the disc diffusion method.

Results: Totally 26 isolates were isolated and 65.3% showed resistance to cefixime, chloramphenicol, clotrimazol, amoxicillin, ampicillin, amikacin and penicillin antibiotics. Of the 32 sample, 61% of the isolates were found to be ESBL producers and highest incidence was found in the age group of 30-45 years. Extended-spectrum beta-lactamase producers belonging to the following species, *E. coli*, *Proteus sp* and *Pseudomonas aeruginosa,* were also multidrug resistant. All three tested ESBL pathogens were highly sensitive to the root extracts of *W. somnifera* and moderately sensitive to leaf extracts. The maximum zone of inhibition of root extracts was 19 ± 0.2 mm against ESBL *E. coli* followed by 18 ± 0.2 mm against ESBL *Pseudomonas aeruginosa* and *proteus sp*.

Conclusions: The present study reveals the potential role of *W. somnifera* against UTI pathogens and it confirms the antibacterial activity of this species against drug resistant clinical pathogens.

Keywords:Withania somnifera; Urinary Tract; Prevalence; Infection

1. Background

Urinary Tract infection (UTI) is the second most common infectious presentation in community medical practice after respiratory tract infections (1). A complicated UTI includes abnormalities of the urinary tract that impede urine flow. The prevalence of urinary tract infections varies with age and sex. The risk factors for UTI involve colonization with a different uropathogen in cases of recurrent UTI, glucosuria and impaired granulocyte function (2). Drug resistance is a natural biological response of microbes involving mutation and cross genetic transfer. It becomes a major problem only when disease-causing organisms develop the ability to fight off disease-curing drugs when these drugs are partially used or used unnecessarily (3). The increasing resistance pattern to a drug creates pressure to switch to a different drug that is more potent than that prescribed previously. It is worth to mention that just as drug resistance is mainly an acquired property; it can also be lost in course of time (4). The aim of this study was to determine the antibiotic susceptibility pattern of uropathogens and their sensitivity towards *W. somnifera*. *Withania somnifera*L. (Dunal) (from the family Solanaceae), which is classically known for its ethnomedicine properties. Many pharmacological studies have been conducted to investigate the properties of *Withania somnifera*as a multi-purpose medicinal agent (5). The plant is used for the treatment of tuberculosis, rheumatism, inflammatory conditions and as a potential anti-tumor agent (6).

2. Objectives

The aim of this study was to evaluate the antibacterial

effect of extracts of *W. Somnifera* on extended-spectrum beta-lactamase (ESBL) pathogens isolated from UTI patients by the stroke method.

3. Patients and Methods

3.1. Sample Preparation

Early morning urine was collected from 32 positive patients who had clinical symptoms of UTI and were from the Trichy region of Tamilnadu. Thirty-milliliter urine samples were collected in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded aseptically and the sediment in the centrifuge tube was homogenized in 100 μL of supernatant. The homogenized suspension was then used for total bacterial count and identification of uropathogens.

3.2. Isolation and Identification of Uropathogens

Uropathogens were identified through culture, microscopy and biochemical tests. MacConkey agar, blood agar, chocolate agar and Oxoid clarity agar media (Himedia Ltd.) were inoculated with 100 μL of the prepared urine samples, and incubated at 37°C for 24 hours. Bacterial identification was done by phenotypic examination followed by Gram staining, and a series of standard biochemical tests were also performed to identify the bacteria of interest (7).

3.3. Antibiotic Susceptibility Testing

The agar disc diffusion assay was used to determine the antimicrobial susceptibilities of uropathogens. The discs used in this study included amoxicillin 30 μg, amoxiclav 30 μg, cefepime (4th) 30 μg, *cefixime* (3rd) 5 μg, chloramphenicol 30 μg, clotrimazol 25 μg, amikacin 10 μg, ampicillin 30 μg, ceftriaxone (3rd) 30 μg, ciprofloxacin 5 μg, gentamycin 10 μg, erythromycin 15 μg and penicillin 10 μg. The diameters of the zones of inhibition for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories according to the National Committee for Clinical Laboratory Standards Institute (8, 9).

3.4. Detection of Extended-Spectrum Beta-Lactamase by the Double Disc Diffusion Method

An amoxicillin-clavulanic acid (20 mg and 10 mg) disc was placed on the center of the plate. Ceftazidime (30 mg), ceftriaxone (30 mg), cefotaxime (30 mg) and aztreonam (30 mg) discs were placed peripherally away from the amoxicillin clavulanic acid disc. After 24 hours of incubation at 37°C, band formation between the amoxicillin clavulanic acid disc and any other disc was considered ESBL positive. The ESBL positive strains were further subjected to phenotypic confirmatory tests using sensitivity discs, which contained third-generation cephalosporins both with and without clavulanic acid. The discs used included cefotaxime (30 mg), cefotaxime and clavulanic acid (30 mg and 10 mg). The differences in the zone of inhibition caused by the cephalosporins alone and when combined with clavulanic acid were recorded and if the difference was 5 mm or more, the strains were confirmed as ESBL-producing strains (10).

3.5. Antibacterial Activity of W. somnifera Against ESBL Producers

Well dried finely powdered roots and leaf were extracted with ethylacetate and concentrated by evaporation. The crude extracts were assayed against ESBL *E. coli*, *Pseudomonas aeruginosa* and *Proteus sp*.; about 100 μL of (5 mg/mL) *W. somnifera* extracts were loaded on sterile discs and were tested against test pathogens. The test pathogens were swabbed on Mueller Hinton agar plates and the sample disc were placed on the center of plates along with a positive control amoxicillin-clavulanic acid and a negative control Ethyl acetate. All the plates were replicated three times and the zone of inhibition was calculated by the mean values.

4. Results

Of the 32 samples, 20 were female and 12 were male and a high frequency of infection was found in the age group between 30-45 years (Table 1). Twenty-six microorganisms have been isolated from urine samples and six different genera were identified. The frequencies of isolates are given in Table 2. Among the 26 isolated UTI pathogens, 42 were ESBL producers belonged to *E. coli* (23%) *Pseudomonas sp* (11.5%) and 3.8% of *Klebsiella sp* and *Proteus sp*. The use of medicinal plants proved to be economical and effective; in addition, they are easily available and safe to use. Root extracts of *W. somnifera* had the highest antibacterial activity against ESBL *E. coli*, (64% relative inhibitory zone) followed by *Pseudomonas aeruginosa (*56 % relative inhibitory zone). The least relative inhibitory zone was 53% against ESBL *Klebsiellasp* and *Proteus* (Table 3). However, antibacterial potentials were also observed against non-ESBL *E. coli*, *Klebsiella*, *Enterococcus* and *Staphylococcus*. Most of the non-ESBL pathogens were highly sensitive to root extract of *W. somnifera* and the maximum activity was 20 ± 0.16 mm against *Klebsiella sp*. This antibacterial activity has been attributed to the presence of some active constituents in the extract. This research finding encourages usage of *W. somnifera* against UTI infection. Further studies such as purification, nuclear magnetic resonance and mass spectrum are required to determine the pharmaceutical value of *W. somnifera*.

Table 1. Frequency of Urinary Tract Infection Among the Collected Samples

Variable	Frequency		Value [a]
	Male	Female	
Age, y			
0-15	0	2	6.25
15-30	2	4	18.75
30-45	6	8	43.75
45-60	3	4	21.87
Above 60	1	2	9.37
Total	12	20	100

[a] Data are presented as %.

Table 2. Frequency of Isolated Pathogens From Urine Samples and Percentage of Extended-Spectrum Beta-Lactamase Producers [a,b]

Name of Organisms	Frequency	Number of ESBL Producer
E. coli	7 (26.92)	6 (23)
Pseudomonas aeruginosa	5 (19.23)	3 (11.5)
Enterococcus sp	4 (15.38)	0 (0)
Klebsiellasp	4 (15.38)	1 (3.8)
Staphylococcus sp	2 (7.69)	0 (0)
Proteus sp	4 (15.38)	1 (3.8)

[a] Abbreviation: ESBL, Extended-Spectrum Beta-Lactamase.
[b] Data are presented as No. (%).

Table 3. Antimicrobial Study of *W. somnifera* Against Extended-Spectrum Beta-Lactamase Pathogens [a]

Test Pathogen (ESBL)	Zone of Inhibition in mm in diameter, mm × Dm				
	Amox/Culv	Ethyl Acetate	Root Extract	Percentage of RIZD	Leaf Extract
E. coli	17	8	19	64	14
Pseudomonas aeruginosa	16	9	18	56	12
Proteus sp	17	9	18	53	15
Klebsiellasp	16	9	18	53	16

[a] Abbreviations: ESBL,Extended-Spectrum Beta-Lactamase;Dm, Diameter; RIZD, Relative Inhibition Zone Diameter.

5. Discussion

In this present investigation it was observed that UTIs are more prevalent in the age group of 30-45 years and are most frequently found in female cases. Among the isolates 15 (58%) isolates were non-ESBL and highly sensitive to cefepime, cefixime, chloramphenicol and clotrimazol. Urinary tract infection is more prevalent in females and there has been many previous reports on this subject (11). In this research, the antibacterial study showed that the root extracts of *W. somnifera* showed potent antibacterial activity against all ESBL producers. The leaf extracts showed maximum zone of inhibition against ESBL *Klebsiellasp* (16 mm) and least inhibitory activity against *Pseudomonas aeruginosa (*12 mm zone of inhibition). Antimicrobial activity of methanolic extracts of *W. somnifera* against *B. subtilis*, *E. coli*, *P. Fluorescens* and *S. aureus* has been reported previously (12). Aqueous extract of *W. somnifera* was more effective against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus* earlier reported by Jaina and Varshney (13).

Authors' Contributions

Abdul M. Kapur carried out the research and prepared the thesis under the guidance of Dr Ahmed S. john.

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