WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 2, 809-816. Research Article ISSN 2277– 7105

ISOLATION AND BIOCHEMICAL IDENTIFICATION OF URINARY TRACT INFECTING BACTERIAL PATHOGENS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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Article Received on
22 Nov. 2017,
Revised on 13 Dec. 2017,
Accepted on 02 Jan. 2018
DOI: 10.20959/wjpr20182-10667

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ABSTRACT

Urinary tract infection is one of the most common infectious diseases in human population. Nowadays UTI has become difficult to treat because of an increasing drug resistance. The main aim of the present study was to determine the bacterial pathogens of UTI and to find out the antibiotic sensitivity pattern of Isolated pathogens. In the present study, totally 201 uropathogenic isolates were isolated from UTI infected patients. Out of 201 uropathogenic isolates, 109 from uncomplicated UTI, 76 from complicated UTI and 16 from asymptomatic bacteriuria. Among the 109 uropathogenic isolates from uncomplicated UTI, 78.9% were *E.coli*. Out of 76 isolates from complicated UTI, 76.3% were *E.coli*. 100% uropathogenic *E.coli* isolates from asymptomatic bacteriuria. Maximum zone of inhibition (38 mm) was recorded while using ciprofloxacin antibiotic against uropathogenic *E.coli*.

KEYWORDS: Urinary tract infections, antibiotic sentivity, antimicrobial drugs.

INTRODUCTION

A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract become infected after bacteria overcome the structures' strong natural defenses. Despite these defenses, UTIs are the most common of all infections and can occur at any time in the life of an individual. Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients, and can lead to significant mortality.[1] UTI account for a large proportion of antibacterial drug consumption and have large socio-economic impacts.[2] UTIs refer to the presence of microbial pathogens within the

urinary tract and it is usually classified by the infection site:- bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria) and also can be asymptomatic or symptomatic, UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated," whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic.[3,4]

Treatment of UTI is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.[5] The prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide due to aberrant use of antibiotics in practice.[6,7] Distribution of urinary pathogens and their susceptibility to antibiotics varies regionally so it becomes necessary to have knowledge of distribution of these pathogens and their susceptibility to antibiotics in a particular setting.[8,9] The aim of this study was to determine bacterial etiologic agents responsible for urinary tract infection and to evaluate their *in vitro* susceptibility pattern to commonly used antimicrobial agents in a private practise set up. This study is important for clinicians in order to facilitate the effective treatment and management of patient with symptoms of urinary tract infection.

MATERIALS AND METHODS

Isolation and identifications of UTI pathogens

A study was planned to determine the etiological bacterial pathogens of the UTI and to determine the antibiotic sensitivity pattern of pathogens isolated. Total 201 samples of urinary tract infection (UTI) outpatients, were collected during this study period. For collection of urine samples patients were advised to collect a clean catch midstream urine specimen in a sterile, wide mouthed leak proof container and bring to the laboratory as early as possible.

The urine samples of UTI patients were collected in Staurt's transport medium and then bring to laboratory for further studies. For the isolation of urinary tract infecting bacterial pathogens, a loopfull of urine samples were streaked on the surface of nutrient agar, Mac Conkey agar, blood agar and chocholate agar plates and incubated at 37^{o}C for 24 hours. Next day, the individual colonies were picked and identified on the basis of their morphological, gram staining and biochemical characteristics.[10,11]

Antibiogram pattern

The antibiogram pattern of UTI pathogens was carried out on Mueller Hinton Agar by Kirby Bauer's disk diffusion method[12] using antibiotic disks from Himedia, Mumbai. The following antibiotics such as amoxicillin (20µg), ampicillin (10µg), ceftazidime (30µg), cephalexin (30µg), ciprofloxacin (5µg), co-trimoxazole (25µg), cefotaxime (30µg), cefuroxime (30µg), nitrofurantoin (300µg), gentamicin (10µg), nalidixic acid (30µg), trimethoprim (5µg), chloramphenicol (30µg), streptomycin (30µg), sulfisomidine (250µg) and tetracycline (30µg) were used for the experiment.

RESULT AND DISCUSSION

Isolation and identifications of UTI pathogens

A total of 201 patients were further subdivided in to uncomplicated urinary tract infection (109 patients), complicated urinary tract infection (76 patients), and those with asymptomatic bacteriuria (16 patients). Table 1 revealed that the subgroup of patients with uncomplicated urinary tract infection (N=109), majority of these yielded *Escherichia coli* (78.9%) followed by *Staphylococcus* sp. (7.3%), *Klebsiella* sp. (7.3%) and *Proteus* (6.4%). In the patients with complicated urinary tract infection (N=76), the most common organism obtained was *Escherichia coli* (76.3%) followed by *Klebsiella* sp. (15.8%) and *Enterobacter* sp. (7.9%). The organism isolated in patients with asymptomatic bacteriuria (N=16) is *Escherichia coli* (100%). The present study coincides with earlier studies[13,14] which showed 61.3% and 59% *E.coli* respectively. Earlier studies[14,15] reported 14.72% and 11.6% cases *Klebsiella spp.* from urine sample in their studies, present study also coincides with their studies. The isolated UTI pathogens were identified using biochemical tests (Table 2). The most common organism isolated from 201 patients is a gram negative bacillus, *E.coli.* It has been demonstrated through serologic testing that the strains of *E.coli* affecting the urinary tract possess a variety of virulence characterisitics that facilitate intestinal carriage, persistence in the vagina and then ascension and invasion of the anatomically normal urinary tract. Complicated urinary tract infections are those that occur in a patient who has a functionally, metabolically, or anatomically abnormal urinary tract or that are caused by pathogens that are resistant to antibiotics. The clinical spectrum ranges from mild cystitis to life threatening urosepsis. Early study[16] classified into those with structural abnormalities, metabolic or hormonal abnormalities, impaired immune response and those caused by unusual pathogens. Fluoroquinolones are preferred as the drug of choice for the treatment of mild to moderate complicated urinary tract infection. *Escherichia coli* are the most common cause of urinary

tract infection in diabetics according to literature. Others like *Klebsiella* and *Proteus* are also common.

Table 1: Uropathogenic organisms isolated from Urinary Tract Infected patients.

Organisms	Uncomplicated UTI	Complicated UTI	Asymptomatic bacteriuria
E.coli	86 (78.9%)	58 (76.3%)	16 (100%)
Staphylococcus sp.	8 (7.3%)	-	-
Klebsiella sp.	8 (7.3%)	12 (15.8%)	-
Proteus sp.	7 (6.4%)	-	-
Enterobacter sp.	-	6 (7.9%)	-

Table 2: Biochemical characteristics of isolated UTI pathogens from UTI patients.

Characteristics	*E.coli*	*Klebsiella* sp.	*Enterobacter* sp.	*Proteus* sp.	*Staphylococcus aureus*
Gram staining	-	-	-	-	+
TSI					
Slant	A	A	A	K	A
Butt	A	A	A	A	A
Gas	G	G	G	G	-
H_2S	-	-	-	-	-
Mannitol	Acid	Acid	Acid	-	-
Motility	Motile	Non motile	Motile	Motile	Motile
Indole	-	-	-	+	-
Methyl Red	+	-	-	+	-
V.P.test	-	+	+	-	-
Citrate test	-	+	-	-	-
Urease test	-	+	-	+	-
Oxidase test	-	-	-	-	-
Catalase test	-	-	-	+	+

+ -Positive; K- alkaline; A -acid; G –Gas.

Antibiogram Pattern

Sixty UTI isolates from UTI patients were subjected for antibiogram pattern and the obtained results are provided in Table 3. It showed that all the isolates were sensitive to amoxicillin (100%). Twelve isolates were moderately sensitive to tobromycin. Twenty eight isolates were resistant to streptomycin antibiotic. Followed by this, twenty four isolates were also resistant to cefotaxime. By using this antibiotic 36 strains were sensitive to tetracycline antibiotic. Drug of choice would be cotrimoxazole (160/800 mg) twice daily; however these should be used with caution in patients taking oral hypoglycemic agents because it can potentiate the hypoglycemic effects of these drugs. Physiologic changes of the collecting system have the greatest impact on bacteriuria in pregnancy. Hormonal factors may also play a role. Because most symptomatic UTIs develop in women with bacteriuria early in pregnancy, treatment of

bacteriuria is undertaken to prevent symptomatic infections. The initial drug of choice for these infections is amoxicillin; however this was not shown to be effective in the present study. Cotrimoxazole, nitrofurantoin, have also been recommended. Short course therapy is given after which a repeat urine culture is done after one week and followed monthly until delivery. Early studies[17] revealed the infection rate due to *E. coli* in pregnant women of North Karnataka region and their resistant pattern to different antibiotics. Early study [18] showed that *E. coli* isolates were the predominant pathogens and showed increasing resistance pattern to the commonly prescribed drugs in private practice that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs.

Table 3: Antibiogram pattern for UTI pathogens from Urinary Tract Infected patients (N=60).

Antibiotics	Disc Potency	Sensitive	Moderately sensitive	Resistant
Amoxicillin	30 mcg	60	-	-
Ampicillin	10 mcg	44	-	16
Ceftazidime	30 mcg	48	4	8
Ciproflaxacin	5 mcg	52	8	-
Co-trimoxazole	1.25 mcg	56	-	4
Cefotaxime	30 mcg	36	-	24
Cefuroxime	30 mcg	36	8	16
Nitrofurantoin	300 mcg	48	-	12
Gentamicin	10 mcg	52	-	8
Nalidixic acid	30 mcg	48	4	8
Chloramphenicol	30 mcg	48	8	4
Streptomycin	10 mcg	32	-	28
Tetracycline	30 mcg	36	8	16
Tobramycin	10 mcg	30	12	18

The percentage (%) of sensitivity and susceptibility pattern for uropathogenic *E.coli* isolates from UTI patients is predicted in Table 4. It explained that 100% sensitivity was recorded while using amoxicillin as antibiotic. Followed by this, 93.3% sensitivity was recorded by using co-trimoxazole antibiotic against uropathogenic *E.coli*. 46.7% of uropathogenic *E.coli* isolates were susceptible to tetracycline. Followed by this, 40% of uropathogenic *E.coli* isolates were susceptible to cefotaxime and 26.7% were susceptible to ampicillin, cefuroxime and tobromycin drugs. For empirical therapy in patients with complicated urinary tract infection, amoxicillin –clavulanic acid and fluoroquinolones showed a broad spectrum of antimicrobial activity covering most expected pathogens and achieve high levels in the urine and urinary tract tissue. Resistance however has been noted in some patients and shift to a more potent antibiotic guided by susceptibility testing could be done in treatment failures.

Amoxicillin –clavulanic acid can be used in the empiric treatment of asymptomatic bacteriuris. Increased resistance has been noted with amoxicillin, which is initially the drug of choice. Nitrofurantoin, oral cephalosporin, or trimethoprim-sulfamethoxazole may also be used.

Table 4: Percentage (%) sensitivity pattern for Uropathogenic *E.coli* isolates of Urinary Tract Infected patients (N=60).

Antibiotics	Disc Potency	Percentage sensitivity (%)	Susceptibility percentage (%)
Amoxicillin	30 mcg	100	-
Ampicillin	10 mcg	73.3	26.7
Ceftazidime	30 mcg	80.0	13.3
Ciproflaxacin	5 mcg	86.7	-
Co-trimoxazole	1.25 mcg	93.3	6.7
Cefotaxime	30 mcg	60.0	40.0
Cefuroxime	30 mcg	60.0	26.7
Nitrofurantoin	300 mcg	80.0	20.0
Gentamicin	10 mcg	86.7	13.3
Nalidixic acid	30 mcg	80.0	13.3
Chloramphenicol	30 mcg	80.0	6.7
Streptomycin	10 mcg	53.3	6.7
Tetracycline	30 mcg	60.0	46.7
Tobramycin	10 mcg	50.0	26.7

CONCLUSION

The most common urinary tract infection causing pathogen in this study is *E.coli*. As drug resistance among bacterial pathogens is a developing process, regular surveillance and monitoring is necessary to provide physician's knowledge on the updated and most effective empirical treatment of urinary tract infections. In order to prevent or decrease resistance to antibiotics, the use of antibiotics should be kept under supervision, should be given in appropriate doses for an appropriate period of time. In addition the sensitivity patterns to other testing antibiotics have been decreasing due to uncontrolled abuse of the available drugs. A strong policy to avoid over counter sale of drugs and encouraging for multi centres periodic studies to win the battle against resistant uropathogens.

ACKNOWLEDGEMENTS

The authors are grateful and thank V.H.N.S.N. College (Autonomous) Managing Board, Virudhunagar for providing facilities to do research.

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