Pathogenic Identification and Antibiotic Susceptibility of the Microorganisms Isolated from Urine Samples of UTI patients

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Introduction

Urinary tract infection (UTI) is the infection that occurs in the urinary tract by the invasion of pathogenic bacteria, fungi, virus etc. under some circumstances only. Some common pathogenic bacteria associated with UTI include Escherichia coli, Proteus spp., Streptococcus spp., Staphylococcus spp., Pseudomonas spp. In the ongoing research work, about 30 UTI patients were selected randomly to detect the pathogen responsible for causing urinary tract infection and also to demonstrate their drug resistant traits. Among the 30 samples, about 14 samples were found to harbor Proteus spp. (1×104 cfu/ml to 1×105 cfu/ml). Escherichia coli was found to be the second predominant agent for UTI and recovered from 13 samples. Among the rest 3 samples, Pseudomonas spp. was found in 2 samples and Klebsiella spp. was found in one sample. Identified isolates showed resistance against different ranges of antibiotics. The study findings revealed the fact that emerging drug resistance of different pathogenic bacteria could become the major difficulties in the treatment of infectious diseases like UTI.

Keywords: Urinary tract infection; Pathogenic bacteria, Antibiotic resistance

Urinary tract infection (UTI) is a common global problem and one of the major causes for which patients seek for medical treatments. Approximately 250 million people world-wide get infected with UTI. Females are more prone to UTI because of the easier and shorter access from the external of the urethra to the urinary bladder than the males who have much longer urethra. Females also get UTI easily during sexual intercourse and child birth. The rate of UTI depends on age, sex, immune status, catheterization etc. Urinary tract infections are mainly caused by bacteria and occasionally by fungi and viruses. Most common bacteria causing such infections include *Escherichia coli*, *Klebsiella*spp., *Proteus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Neisseria* spp. etc. Despite of many antibacterial mechanisms in the urinary bladder to have infection, the uropathogenic bacteria can invade such mechanisms and colonize in the urinary tract to start an infection. Different tests are in use to detect UTI such as urinalysis, Gram staining, urine culture and so on.

The treatment starts with the result of antibiotic resistance of the identified pathogen from urine sample of the patient. The physician prescribes the susceptible antibiotics from the result chart but the condition gets worse when patients use uncontrolled dosage without becoming aware of the drug resistant threats.Immuno-compromised patients are at a great risk of UTI and the drug resistant traits of the pathogens makes the phenomenon even more complicated. HIV infection, diabetes, patients undergoing surgery, organ transplant patients can easily caught by UTI.

In the current study 30 patients were randomly selected seeking medical facilities and supposed to have urinary tract infection. Pathogenic bacterial identification along with their count was determined in this study. In the later part of the study, antibiotic drug susceptibility test was performed for the pathogenic isolates collected from the individual patients. Sensitive, moderate and resistant antibiotic drug list was prepared for the pathogenic isolates separately to get complete array of drug susceptibility result which will help to treat the patients with the best suited susceptible antibiotic.

MATERIALS AND METHODS

Study population and sampling. Suspected urinary tract infection (UTI) patients of various ages (ranging from 14 years to 66 years old) seeking for medical treatment were considered as a study population in this study. About 30 patients were randomly selected who came for microbiological analysis in the Jaipur and Kota region. The study was conducted from March,

2021 to May, 2021. Urine samples were collected aseptically from the mid stream in the sterile bottles provided by the test centers and immediately transferred to different culture media. Standard laboratory procedures were strictly maintained to avoid any hazards and transmissions to healthcare workers and to others.

Determination of pathogenic bacteria responsible for the urinary tract infection. After collection of urine samples, they were inoculated onto agar plates and CLED (cysteine-, lactose- and electrolyte-deficient) agar plates to detect mostly Gram negative bacteria (14-16). An aliquot of 10μl sample was spread over both the types of agar plates and incubated at 37 oC for 24 hours. After incubation period, the colonies were enumerated and subjected to biochemical identification.

Antibiotic susceptibilityity of the isolated pathogens. Pathogenic bacteria found in the urine samples were further subjected to determine their antibiotic sensitivity patterns against the commonly prescribed antibiotics. About 11 antibiotics such as gentamicin (GEN, 10μg), cephradine (CED, 30μg), cefuroxime (CXM, 30μg), ceftadizime (CAZ, 30μg), amikacin (AMK, 10μg), nitrofurantoin (NIT, 300μg), ciprofloxacin (CIP, 05 μg), levofloxacin (LE, 5 μg), rifampin (RIF, 05 μg), ceftriaxone (CTR, 30 μg), cotrimoxazole (COT, 25 μg) were selected to be tested for this study. At first, Mueller Hinton agar plates were used to make lawn of the isolated pathogenic bacterial culture and the selected antibiotic discs were placed over the lawn culture. Afterwards, the plates were incubated at 37 oC for 24 hours. Following incubation, the plates were observed for the zone of inhibition around the antibiotic discs.

RESULTS AND DISCUSSIONS

Urinary tract infection has remained to be one of the prominent medical complications worldwide. Urinary tract is usually sterile but accidental bacterial invasion can occur causing upper and lower urinary tract infection which can be both symptomic or asymptomic. Gram negative bacteria *Escherichia coli* and *Klebsiella*spp. are found to be the most common causes of UTI. *Staphylococcus aureus*and *Klebsiella*spp. has also been reported in the urine samples of UTI patients. In the present study, the most prevalent pathogenic bacteria were *Proteus* spp. and

Escherichia coli which was found to be present in 14 samples and 13 samples, respectively out of 30 samples ranging from 104 cfu/ml to 105 cfu/ml. *Pseudomnas*spp. was found to be present in 2 samples (1.5x104 cfu/ml and 2.2x105 cfu/ml) and *Klebsiella*spp. was present only in one sample (1.8x104 cfu/ml).

In the later part of the study, microbial resistance and sensitivity towards the commonly consumed antibiotics were experimented to understand the current conditions of the medical treatments and the risks caused by the drug resistance bacteria. This approach helps the physicians to prescribe the patients to use sensitive antibiotics with appropriate dosage. But the alarming situation is that the rapidly increasing drug resistance has made the treatment of patients a big challenge. More and more pathogenic bacteria are becoming resistant and seeking and developing new sensitive drugs is a must to recover such conditions.

Bacteria can become resistant by following a variety of mechanisms which help them to avid the deleterious effects of antibiotics. Mutation is a common way of developing drug resistance. *Neisseria gonorrhoeae*shows resistance towards cefixime and ceftriaxone caused by ribosomal mutation. *Pseudomonas aeruginosa*has multidrug efflux mechanism to survive in the presence of antibiotics. Methicillin resistant *Staphylococcus areus*is a common pathogen in the whole world. Many deaths have been reported due to methicillin resistant *S. aureus.* Multidrug resistant pathogenic bacteria cause serious health problems in the immune-compromised patients which encourages secondary and more fatal infections. Drug resistant genes can be carried into other susceptible bacteria by plasmid, bacteriophage, transposons etc. If these elements are absent, resistance can also be occurred by random chromosomal mutations.

In our current research, multiple drug resistance were documented (Table 2) in cohort with the previous studies. 10 *Proteus* spp. isolates were found to be sensitive towards CTR, COT, NIT, GEN, AMK, LE, CIP and RIF antibiotics (Table 2). Among these antibiotics NIT, RIF and AMK showed better susceptibility than other antibiotics by showing larger clear zones in the Mueller Hinton agar medium. CED and CXM antibiotics were showed to be moderate and this indicates that these two antibiotics are going to be resistant in the upcoming days. One sample (sample no. 19) showed moderate activity for RIF. *E. coli* which wass the second predominant pathogen causing UTI in this study (found in 13 samples out of 30) showed susceptibility towards LE, GEN, NIT, AMK, CTR, CIP, CXM and RIF. All of the *Proteus* spp. were resistant to CAZ, COT

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etc. *E. coli* from sample no. 23 showed to most susceptibility towards most of the antibiotics and least resistance (resistant to 2 antibiotics only- CED, RIF). CTR, LE and AMK, GEN, NIT were best suited in killing *Pseudomonas* spp. from sample no. 18, respectively. *Klebsiella*spp. found only in sample no. 13 was sensitive to AMK, RIF, LE and resistant against GEN, CAZ, CIP, NIT, CXM, CED, CTR, COT (Table 2).

TABLE 1. Pathogenic microbial load (cfu/ml) in urine samples

Sample No	Microbial load (cfu/ml)			
	Escherichia coli	*Proteus* spp.	*Klebsiella* spp.	*Pseudomonas* spp.
01	1.1×10^5	0	0	0
02	1.0×10^4	0	0	0
03	1.7×10^4	0	0	0
04	0	1.4×10^4	0	0
05	1.5×10^5	0	0	0
06	0	1.3×10^5	0	0
07	0	2.0×10^4	0	0
08	0	2.2×10^4	0	0
09	0	2.7×10^5	0	0
10	2.0×10^4	0	0	0
11	1.9×10^5	0	0	0
12	0	3.7×10^4	0	0
13	0	0	1.8×10^4	0
14	0	2.4×10^5	0	0
15	0	2.2×10^5	0	0
16	3.0×10^5	0	0	0
17	0	1.8×10^5	0	0
18	0	1.5×10^4	0	0
19	0	0	0	1.5×10^5
20	2.2×10^4	0	0	0
21	3.1×10^5	0	0	0
22	0	1.3×10^5	0	0
23	0	1.5×10^5	0	0
24	2.5×10^4	0	0	0
25	0	2.0×10^4	0	0
26	0	1.4×10^5	0	0
27	0	0	0	2.2×10^5
28	1.7×10^5	0	0	0
29	1.7×10^5	0	0	0
30	1.3×10^4	0	0	0

TABLE 2. Antibiotic susceptibility pattern of the pathogenic bacterial isolates

Sample No	Bacterial isolate	Antibiotic Susceptibility from urine sample		
		Sensitive	Moderate	Resistant
1	E. coli	LE+		GEN,CAZ,CIP,NIT,AMK,CXM,CED,CTR,COT,RIF
2	E. coli	GEN+	COT	CAZ,CIP,LE,NIT,AMK,CXM,CED,CTR,,RIF
3	E. coli	GEN+		CAZ,CIP,LE,NIT,AMK,CXM,CED,CTR,COT,RIF
4	Proteus spp.	LE+ GEN+		CAZ,CIP,NIT,AMK,CXM,CED,CTR,COT,RIF
5	E. coli	LE+ GEN+ NIT+++	CED	CAZ,CIP,AMK,CXM, CTR,COT,RIF, NIT
6	Proteus spp.	LE+ GEN+ NIT+++ CAZ+++	CED, CXM	CIP,AMK,CTR,COT,RIF
7	Proteus spp.	LE,CIP	CXM	GEN,CAZ,NIT,AMK, CED,CTR,COT,RIF
8	Proteus spp.	LE,NIT,AMK GEN		CAZ,CIP,CXM,CED,CTR,COT,RIF
9	Proteus spp.	NIT,LE,CIP	RIF	GEN,CAZ,AMK,CXM,CED,CTR,COT
10	E. coli	AMK,NIT,RIF	COT	GEN,CAZ,CIP,LE,CXM,CED,CTR
11	E. coli	GEN,AMK,CTR		CAZ,CIP,LE,NIT,CXM,CED,COT,RIF
12	Proteus spp.	AMK++		GEN,CAZ,CIP,LE,NIT,CXM,CED,CTR,COT,RIF
13	Proteus spp.	NIT++,RIF+		GEN,CAZ,CIP,AMK,CXM,CED,CTR,COT
14	Proteus spp.	NIT,RIF		GEN,CAZ,CIP,AMK,CXM,CED,CTR,COT
15	E. coli	AMK,NIT,GEN,CTR		CAZ,CIP,CXM,CED,COT,RIF
16	Proteus spp.	NIT,LE,CIP		GEN,CAZ,AMK,CXM,CED,CTR,COT,RIF
17	Proteus spp.	AMK+		GEN,CAZ,CIP,NIT,CXM,CED,CTR,COT,RIF,LE
18	Pseudomonas spp.	CTR,LE		GEN,CAZ,CIP,NIT,AMK,CXM,CED,COT,RIF
19	E. coli	CIP,LE		GEN,CAZ,NIT,AMK,CXM,CED,CTR,COT,RIF
20	E. coli	CTR,CXM,AMK, GEN,NIT		CAZ,CED,COT,RIF
21	Proteus spp.	NIT,LE,CIP		GEN,CAZ,CIP,NIT,AMK,CXM,CED,CTR,COT,RIF
22	Proteus spp.	AMK,GEN,NIT		CAZ,CIP,CXM,CED,CTR,COT,RIF
23	E. coli	COT,CIP,LE,CTR,AMK,CAZ,NIT,CXM,GEN		CED,RIF
24	Proteus spp.	LE+		GEN,CAZ,CIP,NIT,AK,CXM,CED,CTR,COT,RIF
25	Proteus spp.	GEN,AMK,NIT		CAZ,CIP,CXM,CED,CTR,COT,RIF
26	Pseudomonas spp.	LE,AMK,CIP,GEN		CAZ,NIT,CXM,CED,CTR,COT,RIF
27	E.coli	NIT,LE,AMK		GEN,CAZ,CIP,CXM,CED,CTR,COT,RIF
28	E. coli	LE,CIP		GEN,CAZ,NIT,AMK,CXM,CED,CTR,COT,RIF
29	E. coli	COT,NIT,LE,RIF		GEN,CAZ,CIP,AMK,CXM,CED,CTR

It is very important to prescribe antibiotics after antibiotic sensitivity test and it should be advised in appropriate dosage. The patients must be instructed correctly to take the medications just as prescribed and should also be informed properly about the deleterious effects of misuse of the antibiotics heading to the drug resistance which not only cause harm to the individual but also the people in surroundings.

CONCLUSION

Though urinary tract infection is a common phenomenon, the treatment procedure is becoming more complicated due to multi drug resistant pathogenic bacteria. Only a few antibiotics are still in susceptible condition. But the resistance toward these antibiotics could be an obvious condition for the future. Development of new drugs is a must and patients should be advised properly to take the antibiotic medication to control the condition of rapidly occurring resistance traits.

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