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Antibiotic resistance pattern in Urinary tract infection patients in Bhopal, Madhya Pradesh, India

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Abstract

Infections including urinary tract infections are among the most common bacterial driven disease in humans, both as community-acquired and healthcare-associated infections. As per World Health Organization (WHO), urinary tract infections are second largest infections to human and responsible for the massive amount of antibiotics consumption. Multidrug-resistant uropathogens are major health threats to both developing and developed nations. The clinical application of new generation antibiotics becomes a challenge with the emergence of MDR, XDR and PDR. In our study, we have profiled and reported major uropathogens in Bhopal city and their diversity. The Escherichia *coli* (more than 60%) remain a major uropathogen associated in UTIs cases in Bhopal. The other uropathogens reported in present study are *Klebsiella,Enterobacter, Pseudomonas* and *Staphylococcus*. The isolated and profiled uropathogen have shown increasing drug resistance with all major commercial antibiotics. Based on the pattern of antibiotic resistance multi-drug resistant uropathogens were classified as MDR, XDR, and PDR.

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Key words: Antibiotics, Multidrug resistance (MDR), Urinary Tract Infection (UTI) and bacterial diversity

Introduction

Urinary tract infections (UTIs) are leading infectious illness worldwide, affected a large number of population yearly (Shah *et al.,* 2004; Wiener *et al.,* 1999).As per WHO reports, UTIs infections are second largest infections to the human and primarily women of all age groups (Perez *et al.,* 2007). The uropathogens associated with UTIs are largely gram-negative bacteria including *Escherichia coli*, *Enterobacter, Proteus* and *Staphylococcus*(Paterson and Bonomo 2005; Pitout and Laupland 2008; Livermore *et al.,* 2007). The UTIs are more common in developing countries and female are easily get infected as the proximity of anatomy. The uropathogens rapidly colonize in the lower urinary tract resulting acute urinary tract infection (Jacoby and Munoz-Price 2005). The uropathogens are capable of biofilm formation that provides an ideal environment for bacterial growth by neutralizing acidic pH of the urethra and vaginal orifice (Niumsup*et al.,* 2008). The major symptoms in UTIs are a lower abdominal cramp, burning sensation, pink color urine with epithelial cells, pus cells and WBC cells and being turbid. The acute UTIs if not treated can result in a serious infection to the urinary bladder and entire renal system (Kidney and ureter) resulting in chronic inflammation. The chronic UTI may lead to infection to genital parts including vagina and cervix. The lactobacilli are predominantly present in the vagina and maintain a slightly acidic pH and prevent any further infections (Zandi*et al.,* 2017). Subsequently, candida infection as a potential vaginal fungal pathogen is a result of chronic UTIs (prolonged) in the majority of women (Ding *et al.,* 2008).

Clinically, acute infections of the urinary tract divided into lower tract infections (urethritis and cystitis) and upper tract infections (acute pyelonephritis, intrarenal and perinephric abscess) (Walsh *et al.,* 2005). Re-infection and relapse are common in women who develop complicated UTI. The management of UTIs both acute and chronic depends on precise diagnosis and the use of antibiotics with the plenty of other medications including diuretics (Mathur*et al.,* 2002). The therapeutic remedy depends on several kind factors including the type of uropathogen, the severity of infection (CFU) and patient's pathological conditions (Karas*et al.,* 1996). The use of antibiotics and associated medicine last for few days to few months. The use of diuretic is quite useful in case of chronic UTIs. During chronic UTI infection to renal system alter renal physiology leading to the alternation of water and salt imbalance (Jonathan 2005; Jarlier*et al.,* 1998). As the UTIs are second largest infection worldwide and a massive amount of antibiotics are being used since many decades leading to bacterial resistance. The concurrent and frequent use of antibiotics (alone and or in combinations of other antibiotics) is prime cause for emergence of drug resistant uropathogens.

Since last few decades, there is increasing evidence that uropathogen have gained resistance to the majority of commercial antibiotics and drug-resistanturopathogen become major health issue (Singha l*et al.,* 2005). It has been well reported that extended spectrum β-lactamase (ESBL) producing uropathogen are capable of surviving in the various antibiotics including amino and ureido

penicillin, oxyimino cephalosporin, and monobactams (Shukla *et al.,* 2004; Supriya *et al.,* 2004). Microbes have several mechanisms in the acquiring resistance against antibiotics and ESBLs are major one reported among uropathogen worldwide. Additionally, environmental factors such as geographical conditions and ethnicity are crucial in developing resistance to bacteria including uropathogens (Lucet*et al.,* 1999; Subha and Ananthan 2002). There are several findings clearly demonstrates countries with humid climate are much prone for UTIs cases. Within India, there has been a history of frequent UTIs cases in the southern part relatively higher than rest of states in India. Andhra Pradesh, Tamilnadu, Karnataka, and Maharashtra are leading in UTI cases reported in last few decades (Cannon *et al.,* 2007).

Now, the UTIs are not only limited to the listed states within India but also spread across the nation. There is not only increasing UTIs cases in Uttar Pradesh, Madhya Pradesh, Chhattisgarh, and Bihar but also there is increase in the diversity of uropathogens (Rodríguez-Bano*et al.,* 2010). The goal of this study was to investigate the prevalence of UTIs in Bhopal city, Madhya Pradesh, India with antibiotic resistance pattern. The study was also associated to investigate the diversity of uropathogen in context with the nature of drug resistance.

Material and Methods

Collection of sample

The study was aimed for a period from February 2015 to February 2016. A total 500 mid-stream urine samples were collected in a sterile container from suspected urinary tract infection patients at Bhopal city, Madhya Pradesh, India. For the present study the samples were collected from both indoor and outdoor patients at Department of Microbiology, Kasturba and Parul hospital, Bhopal City, Madhya Pradesh, India. All the samples were collected from suspected urinary tract infection patients considering all the different age groups and socioeconomically background. Under sterile condition,first-morning clean catch midstream void urine samples were collected and processed under the routine microbiology protocol of urine analysis.

Sample processing

All the consumables used in the sample processing and screening of uropathogenwere procured from Hi-Media. Under the standard microbiological protocol, all the urine samples from suspected urinary tract infection patients were processed and profiled for potential uropathogens. Identification of uropathogenswas madeby gram reactions, colony morphology, biochemical characteristics and growth pattern in selective media. Here, with standard calibrated loop delivering 0.01ml of urine was inoculated on UTI agar, Mac Conkey agar, EMB agar incubated aerobically at 37°C for overnight. If the CFU is more than 10^5 it consider as significant bacteria.

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion on Mueller Hinton agar according to the guidelines of the CLSI. Here, we have used disk diffusion method for the antimicrobial susceptibility of profiled microbes screened from suspected urinary tract infection patients. The disk diffusion method and results were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2006) (Helio*et al.,* 2007). Here, in the study, seventeen antibiotics were used for antibiotic susceptibility analysis of uropathogenic microbial strains includingAztreonam (AT), Norfloxacin (NX), Ofloxacin (OF), Cefpodoxime (CPD), Ciprofloxacin (CIP), Amoxycillin/Clavulanic Acid (AMC), Ampicillin (AMP), Nitrofurantoin (NT), Cefoperazone (CPZ), Gentamycin (GEN), Ceftazidime (CAZ), Piperacillin (PI), Imipenem (IPM), Co-Trimoxazole (COT), Cefuroxime (CXM), Nalidixic acid (NA) and Meropenem (MRP).

Data analysis and statistical

The samples and data were analyzed using with WHONET 5.6 software freely available and recommended by the World Health Organization (WHO). The software along with available database provides an ease to characterize and quantify the epidemiology of urinary tract infections in a given geographical region and population. We have determined the percentage of antibiotic resistance in a given period (2015-2016) and the rates of multi-resistant strains among uropathogen characterized. The multi Resistant strains were divided into MDR (Multiple Drug-Resistant), XDR (Extensively Drug-Resistant) and PDR (Pan drug-resistant) according to the European Centre for Disease prevention and Control (ECPDC).

As per recommendation from ECDPC, if a bacterium develops resistance against at least three different classes of antibiotics referred as MDR, XDR bacteria show resistance against only one class of antibiotics while PDR bacteria are resistant to all classes of antibiotics recommended for treatment and are in clinical use.

Results

Prevalence of urinary tract infections

Among 500 samples collected during 2015-2016, we have reported bacterial growth in 212, evidence for UTIs association while other 298 failed to grow in all recommended growth media. The bacterial pathogens isolated are largely gram negative including *Escherichia coli* (62%), *Klebsiella* (14%), *Enterobacter* (11%), *Staphylococcus* (6%), *Pseudomonas* (4%) and *Proteus* (3%). A detailed distribution of uropathogen isolated and profiled from the urine samples from UTIs patients summarized in table 1.

Antibiotics resistance

The screened and profiled uropathogenswere analyzed for all the major antibiotics for their resistance and relative resistance among uropathogens. Among profiled uropathogen *Escherichia coli* was reported carrying a higher percentage of antibiotic resistance for all the commercial antibiotics. In general *Escherichia coli* isolated from urine samples in present study succeeded in attaining resistance nearly 50%. In our study, *Enterobacter* had shown maximum resistance; AT (100%), CAZ (100%), CPD (90%) and NA (72%). Similarly, *Staphylococcus* had shown resistance for a wide range of antibiotics including COT, AMC, AMP and NA (83%) while more than 60% with other antibiotics such as NIT, CIP, CPD, and CAZ. *Klebsiella*, another major uropathogen reported in higher frequency (next to *E. coli*) in the present study was reported with acquired resistance for a wide range of antibiotics; CPD, AMC and AMP (90%), NIT (85%) and CAZ, COT, CXM (70%). *Staphylococcus* and *Pseudomonas* also have shown resistance to all major antibiotics. A summary of antibiotics resistance and sensitivity against all major antibiotics listed in table 2 and figure 2.

Multidrug resistance (MDR, XDR, PDR)

Profiled uropathogen based on their pattern of drug resistance and using WHONET 5.6 software all drug-resistant bacteria were classified as MDR, XDR, and PDR. Among 212 uropathogens associated in UTIs, the strict MDR were reported 64 in number. The percentage of drug resistance was reported unique in all the major uropathogen as described in table 3 and figure 3. The percentage of drug resistance was reported much higher as 64% total for all the uropathogens. The XDR and PDR percentage in given population was determined as 8.0% and 4.0% respectively. The *Escherichia coli* remain major uropathogen and possess a higher drug resistance among other reported uropathogens. The total MDR percentage for *E. coli* in given population was reported20. 96% while XDR and PDR were reported 6.45% and 1.62% respectively. The other two uropathogens *Klebsiella* and *Enterobacter*were reported with the significant higher drug resistance in the present study. The percentage of drug resistance for *Klebsiella* (MDR (71.42%), XDR (14.28%) and PDR (7.14%)) while in case of *Enterobacter* percentage of MDR (81.81), XDR (9.09%) and PDR (9.09%) remain a slightly higher.

Discussion

The present study concludes diversity of uropathogen associated in acute and chronic urinary tract infection in Bhopal, Madhya Pradesh, India. The major uropathogen reported among urine samples from suspected urinary tract infection patients are *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Staphylococcus* (Minamida *et al.,* 2011). The uropathogen were reported in different percentage as mentioned in table 1. However, *Escherichia coli* remain as major uropathogen and have shown similarities with studies previously carried out. In the present study, *Enterobacter* and *Klebsiella* were reported in higher percentage nearly 14% and 11% respectively which is unique from previous studies carried out in Sothern and North East states of India (Kariuki*et al.,* 2007).

The antibiotic resistance among profiled uropathogens reported significant in given population. Previous studies have shown *E. coli* possesses the capacity to develop resistance to major commercial antibiotics, and here in the presentstudy, we have reported a similar finding (50%). The other uropathogen*Enterobacter* and *Klebsiella* which were present in higher frequency in UTIs patients do possess higher drug resistance in the present study (Table 1 and Table 2). Jenifer et al. 2009 have studied the prevalence of lower urinary tract infection in Southern part of India and present study has shown a significant resemblance in prevalence and diversity of uropathogens (Janifer*et al.,* 2009).

Similarly, Manjula et al. 2013 have demonstrated prevalence and diversity of uropathogens in Karnataka, India (Manjula*et al.,* 2013). A recent finding carried out in 2017, Ranjanet al. in Andhra Pradesh successfully profiled all major uropathogens associated in the UTIs and its reoccurrence (Ranjan*et al.,* 2017). The present study has shown presence of *Escherichiacoli* as universal uropathogen and a significant drug resistance. The UTIs cases were reported more common during pregnancy and in case of high blood sugar level i.e. diabetes mellitus (Eshwarappa*et al.,* 2011). Apart from genetic risk factors (genes and encoded enzymes) several environmental factors are crucial in the onset and reoccurrence of UTIs cases not only in India but also rest of world. The geographical location and climate largely favour growth of selective microbes and hence uropathogens. The humid environmental conditions are driving force behind UTIs cases and reoccurrence. In India Southern and central India has significant larger cases for UTIs (Tanej*aet al.,* 2008). Simultaneously, countries like Bangladesh and Nepal have large number cases of UTIs in last past few decades (Tanej*aet al.,* 2013).

Both, the increasing cases and relapse of UTIs demonstrate the failure of the present of therapeutics intended for clinical use. Majority of uropathogenwas succeeded in acquiring resistance against all class of major antibiotics (Chhetri*et al.,* 2001). The ESBL producing uropathogens are not only capable of developing resistance tothird-generationcephalosporins but also other class of antibiotics too. The complete mechanism is still unknown, but uropathogens are capable of producing enzyme present as separate plasmid and or integrated into thegenome. As enzymes are versatile molecule and possess an affinity for multiple substrates can bind multiple substrates (Verma and Pulicherla; 2016; 2017). The increasing promiscuous nature of enzyme could be a potential mechanism uropathogen have acquired and developed resistance. Now, both *Enterobacter* and *Klebsiella* will be categorized as XDR and PDR in the present study for the geographical location such as Bhopal. The third generation cephalosporin including ceftriaxone, ceftazidime, and cefotaxime was effective for most of uropathogen. Based on antibiotic sensitivity test it is quite clear the third generation cephalosporins are still effective and useful for the management of UTIs.

Conclusion

The present study enlights distribution of uropathogens associated in urinary tract infection in Bhopal, Madhya Pradesh, India. Further, the study concludes behavior of uropathogens against commercial antibiotics. As we have reported here in the present study is that addition to *Escherichia coli*, two other major *Enterobacter* and *Klebsiella* are increasingly involved in urinary tract infection and rapidly acquiring drug resistance. The study also enlights emergence of multidrug-resistanturopathogens as MDR, XDR, and PDR based on pattern and extent of drug-resistant against commercial antibiotics. The present study provides a basis for thesafe use of third-generation cephalosporin and to avoid antibiotic against which uropathogen have developed resistance. The finding also suggests and recommends microbiological diagnosis before use antibiotic as geographical region and ethnicity may alter potential uropathogen and pattern of drug resistance as well.

Conflict of Interest

The author declares no conflict of interest.

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Abbreviations

Double Disc Synergy Test (DDST); Extended Beta-Lactamase (ESBL), Multi-Drug Resistance (MDR); phenotypic confirmatory disc diffusion test (PCDDT), Clinical and Laboratory Standards Institute (CLSI); Aztreonam (AT), Norfloxacin (NX), Ofloxacin (OF), Cefpodoxime (CPD), Ciprofloxacin (CIP), Amoxycillin/Clavulanic Acid (AMC), Ampicillin (AMP), Nitrofurantoin (NT), Cefoperazone (CPZ), Gentamycin (GEN), Ceftazidime (CAZ), Piperacillin (PI), Imipenem (IPM), Co-Trimoxazole (COT), Cefuroxime (CXM), Nalidixic acid (NA) and Meropenem (MRP). Multi Drug Resistance (MDR), Extended Drug Resistance (XDR), and Pan Drug Resistance (PDR).

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Tables

Table 1; Distribution of uropathogens in UTI suspected patients in Bhopal city Madhya Pradesh, India

Uropathogens/Organisms	Total numbers
E. coli	62
Enterobactor	11
Klebsiella	14
Staphylococcus *aureus*	6
Pseudomonas	4
Proteus	3

Table 2; Pattern of antibiotics resistance/sensitivity among uropathogens isolated and profiled from urine samples of UTI patients at Bhopal, Madhya Pradesh

A. ***Klebsiella***

AT	NX	OF	CPD	CIP	AMC	AMP	NIT	CPZ	GEN	CAZ	PI	IPM	COT	CXM	NA	MRP
R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
10/4	5/9	5/9	13/1	6/8	13/1	13/1	12/2	8/6	5/9	11/3	9/5	3/11	9/5	10/4	11/3	3/11
71.42%	35.71%	35%	92.85%	42.85%	92.85%	92.85%	85.71%	57.14%	35.71%	78.57%	64.28%	21.42%	64.28%	71.42	78.57%	21.42%

B. ***E. coli***

AT	NX	OF	CPD	CIP	AMC	AMP	NIT	CPZ	GEN	CAZ	PI	IPM	COT	CXM	NA	MRP
R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
22/40	13/49	41/21	56/06	37/25	54/08	56/08	35/27	47/15	34/28	51/11	52/10	Mar-59	49/13	51/11	56/06	08/54
35%	20%	66%	90%	59%	87%	90%	56%	75%	56%	82%	83%	4%	79%	82%	90%	12%

C. Pseudomonas

AT	NX	OF	CPD	CIP	AMC	AMP	NIT	CPZ	GEN	CAZ	PI	IPM	COT	CXM	NA	MRP
R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
2/2	1/3	2/2	4	1/3	4	4	2/2	1/3	1/4	3/1	2/2	0/4	2/2	2/2	2/2	3/1
50%	25%	50%	100%	25%	1%	100%	50%	25%	25%	75%	50%	0%	50%	50%	50%	75%

D. Staphylococcus

AT	NX	OF	CPD	CIP	AMC	AMP	NIT	CPZ	GEN	CAZ	PI	IPM	COT	CXM	NA	MRP
R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
3/3	2/4	4/2	4/2	4/2	5/1	5/1	4/2	4/2	1/5	4/2	3/3	1/5	5/1	4/2	5/1	1/5
50%	33.33%	66.70%	66.70%	66.70%	83.30%	83.30%	66.70%	66.70%	16.50%	66.70%	50.00%	16.50%	83.00%	66.70%	83.30%	16.50%

E. Enterobactor

AT	NX	OF	CPD	CIP	AMC	AMP	NIT	CPZ	GEN	CAZ	PI	IPM	COT	CXM	NA	MRP
R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
11/0	3/9	5/6	10/1	4/7	5/6	6/5	5/6	3/8	6/5	11	4/7	2/9	5/6	4/3	8/3	2/9
100%	27.20%	45.45%	90.90%	36.36%	45.45%	54.50%	45.40%	27.27%	54.50%	100%	36.36%	18.18%	45.45%	36.36%	72.72%	18.18%

Table 3; Patten of drug resistance in Multi drug Resistance (MDR) in given uropathogens from UTI patients at Bhopal City Madhya Pradesh, India. The pattern of drug resistance was determined bases on European Centre for Disease prevention and Control (ECPDC) guidelines. The uropathogens were classified as MDR, XDR and PDR as per ECPDC guidelines.

Uropathogens	Total No	MDR	%	XDR	%	PDR	%
E. coli	62	13	20. 96	4	6.45	1	1.62
Klebsiella	14	10	71.42	2	14.28	1	7.14
Enterobactor	11	04	81.81	1	09.09	1	9.09
Staphylococcus *aureus*	06	13	36.36	1	16.66	1	16.66
Pseudomonas	04	04	100%	0	00	0	0
Proteus	03	02	66.66	0	00	0	0
Total	**100**	**64**	**64%**	**8**	**8.00%**	**4**	**4.00%**

Figures

Figure 1; Distribution of uropathogens associated in urinary tract infection (UTIs) in Bhopal, Madhya Pradesh.

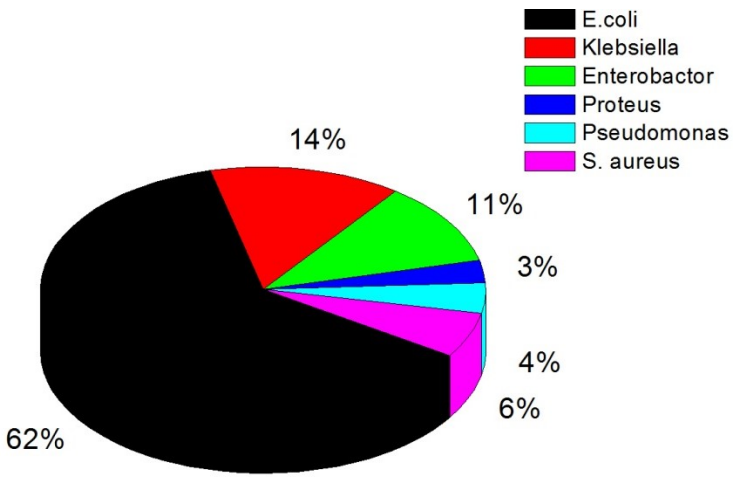

Figure 2; Pattern of antibiotics resistance among uropathogens isolated and profiled from urine sample of UTIs patients from Bhopal, Madhya Pradesh.

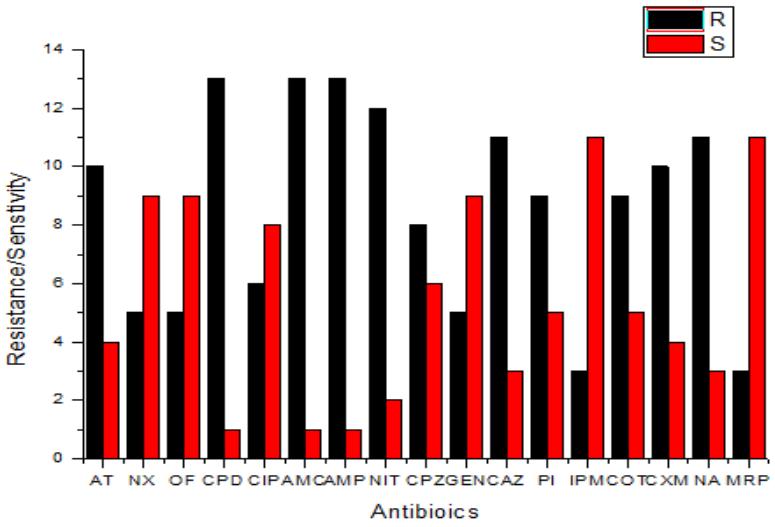

Figure 3 The pattern and prevalence of multidrug resistant uropathogens in given population. The figure demonstrates percentage of MDR as strict MDR, XDR and PDR.