International Journal of Research in BioSciences
Volume 7 Issue 1, pp. (57-63), January 2018
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Prevalence of ESBL phenotype in uropathogens associated urinary tract infections in Bhopal, Madhya Pradesh, India

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(Received December 01, 2017, Accepted December 25, 2017)

Abstract

The uropathogenic microbial strains and associated infectious diseases such as urinary tract infections remain a major infectious diseases threat worldwide. More likely females are much prone to getting UTIs and subsequent pathological consequences due to anatomical specifications. UTIs are ubiquitous with in India with a higher proportion in southern and central India. The emergence of MDR is major health concern associated with infectious diseases. Considering UTIs, and associated pathogens, ESBL producing uropathogenic microbial strains that least studied and reported in central part of India. In this study, we aimed Bhopal city, Madhya Pradesh, India for ESBL phenotype in uropathogenic microbial strains isolated from UTIs patients. In our findings, there is the prevalence of *E. coli* in all uropathogenic microbial strains among the 500 samples studied. Out of these, more than half (60%) were reported resistance for all major antibiotics. Here, from our study, *E. coli* was reported as a major MDR and ESBL (2% of MDR) producing uropathogenic microbial strains associated with onset and progression of infection in Bhopal city.

Keywords: Extended β-lactamase (ESBL), Multidrug resistance (MDR) and Gram negative bacteria, Urinary Tract Infection (UTI)

Introduction

The urinary tract infection remains a major problem worldwide precisely developing countries[1]. The emergence of multidrug resistant (MDR) has further intensified existing problem at globally affecting developed nations too[2]. Asian countries are a major victim for different gram negative uropathogenic microbial strains and on an average, every woman in India and other developing countries have moderate to severe urinary tract infection[3]. The prevalence of urinary tract infection remains high in women due to anatomical specifications. On an average men and women ratio for getting infections with uropathogenic microbial strains and subsequently severe to moderate urinary tract infection estimated 8:1[4]. The common symptoms of a UTI are dysuria, urinary frequency, urgency, suprapubic pain and possible haematuria. There are several gram negative bacteria associated with the onset urinary tract infection including *Escherichia coli, Klebsiella species, Pseudomonas species, Proteus species, Enterobacter, Citrobacter* and *Enterococcus species etc.*[5]. However, considering India and other developing countries *E. coli* remain major uropathogenic microbial strains associated with the onset and progression of the disease. Urinary tract infections (UTIs) are the second most commonly diagnosed infectious illness worldwide, with about 150 million diagnosed yearly[6]. Gram-negative bacilli are the most common pathogens that cause UTIs in both men and women with a ratio of 1:2, with *Escherichia coli* being the most prevalent type accounting for 75-90% of UTIs[7].

Now, in the present scenario, clinical findings have demonstrated a new challenge associated with urinary tract infection. The frequent use of antibiotics allows uropathogenic microbial strains to acquire resistant to existing antibiotics[8,9]. The multidrug resistant uropathogenic microbial strains are quite difficult to cure and management of urinary tract infections. The most significant finding in last two decades suggested that there are emergence uropathogenic microbial strains with a capacity of producing beta lactamase. The enzymes are highly robust in catalyzing biochemical reactions and highly specific for their substrate[10,11]. Hence, genes encoding such enzyme selectively provide a resistance to the organism. Further, extended beta-lactamase producing uropathogenic microbial strains have been reported across the India and more predominantly in the Southern and middle state of India[12]. These uropathogenic microbial strains capable of producing ESBLs are effective in breaking β lactam ring and escape from antibiotics spectrum[13]. There also seems to be a discrepancy between geographical regional resistances and susceptibilities of ESBL organisms. The enzyme is responsible for resistance to amino and ureido penicillin, oxyimino cephalosporin, and monobactams, but not to 7-α-substituted β-lactam[14]. Certain patients have been found to be more susceptible to these infections such as patients with numerous comorbidities, diabetes, live in nursing homes, frequent use of antibiotics, recurrent UTIs, older age, and male sex[16]**.** Here, in the study, we aimed to profile prevalence of ESBLs producing uropathogenic microbial strains in Bhopal, Madhya Pradesh.

Materials and Methods

Collection of sample

To isolate uropathogenic microbial strains, a total 500 samples were collected from patients both male and female suspected of urinary tract infection at Bhopal City, Madhya Pradesh, India during the period of February 2015 to February 2016. The samples collected for the study includes both indoor and outdoor patients across various clinical pathologies and hospitals from the Bhopal city, Madhya Pradesh, India.

Screening of microbial strains

Using standard microbiological procedure all the urine samples collected from suspected urinary tract infection patients were profiled for different uropathogenic microbial strains. Here, we have used standard culture media in the screening of uropathogenic microbial strains including Blood agar, Mac Conkey agar, Muller Hinton agar, Antimicrobial susceptibility discs and other chemicals. All these consumables were procured from Hi-Media, Mumbai, India.

Antimicrobial susceptible microbial testing

The antimicrobial susceptibility of profiled microbes from susceptible urinary tract infection patients was carried out using disk diffusion method and results were interpreted according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2006)**[16]**. A total 17 antibiotics were used here in the study for antibiotic susceptibility analysis of uropathogenic microbial strains including AT, NT, OF, CPD, CIP, AMC, AMP, NIT, CPZ, GEN, CAZ, PI, IPM, COT, CXM, NA and MRP.

Detection of ESBLs producing microbes

There are several methods for the detection of ESBLs producing bacterial strains including Combination Disc Test (CDT) and/or Double-Disc Synergy Test (DDST). Here, in this study, extended β lactamase producing strains were screened using modified double-disc synergy test (PCDST), manual methods using presumptive and confirmatory tests[17]. An agar plate was inoculated with the selected microbial culture and incubated overnight at 37C. Four third generation Cephalosporin standard antibiotics (Ceftazidime, Cefotaxime, Ceftriaxone, and Cefepime with the concentration of 30 µg each) were placed at the center to center distances of 30 mm apart from an Amoxicillin-Clavulanic acid disc (20/10 µg). A clear extension of greater than 5 mm inhibition zone between any of the four Cephalosporins towards the disc containing Clavulanic acid was interpreted as positive for ESBL production.

While in the case of DDST, the distance between the discs is critical and 20 mm center-to-center has been found to be optimal for cephalosporin 30 µg discs. We have further confirmed all the strains for ESBL production by using the PCDDT, as recommended by the CLSI. Here, ceftazidime (30 µg) disc alone and in combination with clavulanic acid (ceftazidime + clavulanic acid discs) were applied to Mueller Hinton Agar (MHA) plates inoculated with selected strains. The confirmation of ESBL producing strains is based on an increase in the zone of inhibition by 5mm in combination disc compare to ceftazidime disc alone.

Results and Discussion

In the present study was carried out at Department of Microbiology, Sri Satya Sai Women College, Bhopal, and Madhya Pradesh, India. A total 500 samples from Kasturba Hospital and Parul Hospital, Bhopal were collected from suspected UTI patients (both indoor and outdoor) and processed for detection and screening of uropathogens. Among isolated uropathogens, major UTIs associated bacteria are *E. coli* (68.18%), Klebsiella (13.64%), Enterobacter (7.58%), Pseudomonas (6.06%) and rest Proteus and *S. aureus*. (Figure 1 and table 1) Here, we have studies uropathogens wise antibiotic resistance and test clearly demonstrated maximum resistance for *E. coli* shown by NT 95% and minimum for NX 34%. Similarly, for *Klebsiella* shows maximum resistance for AMP (89%) and minimum for COT (45%). In the case of Proteus maximum antibiotic resistance was reported for AMP (56%) and least in the case of NX (13%). However, Enterobacter had shown a similar pattern i.e. maximum antibiotic resistance for the AMP (80%) and minimum for NP (23%). (Figure 2 and table 2).

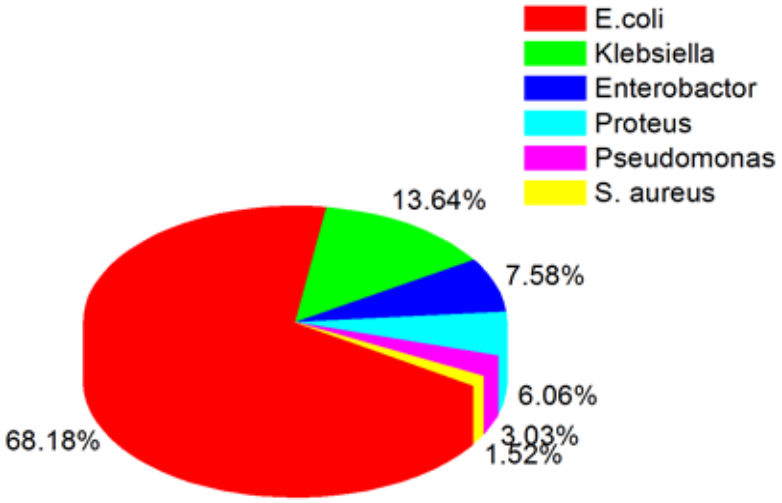

Figure 1: Prevalence of Uropathogens in UTIs. The study concludes *E. coli* as a major uropathogen associated in urinary tract infection

Table 1: The table demonstrates a statistics of various uropathogens associated in the onset and progression of urinary tract infection. Among the identified uropathogens, the *E. coli* remains a major uropathogens accounting nearly half, 45%

S. No.	Organism	Total No
1.	*E coli*	45
2.	*Klebsiella*	9
3.	*Proteus*	5
4.	*Enterobactor*	4
5.	Pseudomonas	2
6.	Streptococcus aurous	1

Table 2: Antibiotics sensitivity pattern of uropathogens against selected antibiotics. The table enumerates gram negative uropathogens having prevalence in the onset and progression of urinary tract infection

S. No.	Organism	Antibiotics				
		AMP	COT	CIP	NT	NX
1.	*E. coli*	92	72	70	95	34
2.	*Klebsiella*	89	45	56	66	65
3.	*Proteus*	56	45	23	23	13
4.	*Enterobactor*	80	45	23	55	35

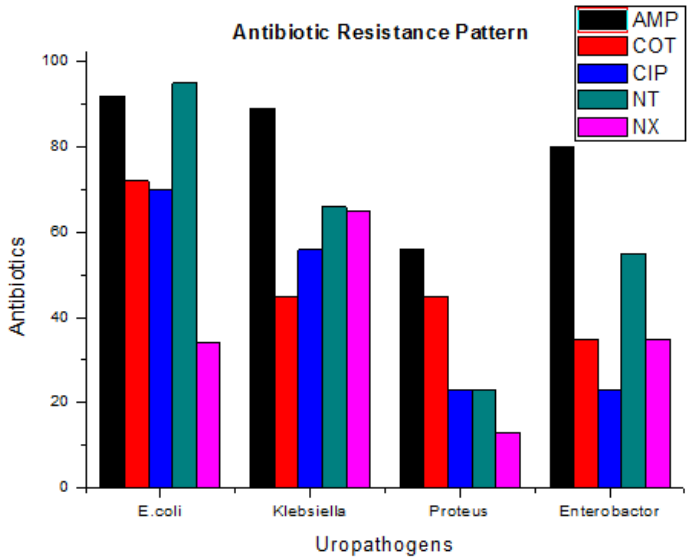

Figure 2: Antibiotic resistance pattern of uro-pathogen isolated from suspected UTI patients

The resistant uropathogens were further examined for the multidrug resistance (MDR) and MDR isolates examined in this study demonstrate that *E. coli* alone account for more than 59% and rest are Klebsiella (10%) and Enterobacter (4%) (Figure 3 and table 3). Randomly we have selected forty isolates for ESBL test using both DDST and PCDDT (Figure 4). *E. coli* was the most common ESBL producer which was found in selected isolates followed by *Klebsiella* and *Enterobacter. E. coli* showed the maximum ESBL prevalence in UTIs patients' urine samples collected from Bhopal City, Madhya Pradesh. The maximum ESBL production was seen in urine samples and E coli was reported as a major uropathogen. (P value <0.05). The *E. coli* was determined and remains a major uropathogen capable of ESBL production and has resistance for a wide range of antibiotics. Similarly, other two uropathogen often reported including Klebsiella and Enterobacter were also reported for ESBL phenotype.

Table 3: The table demonstrates MDR isolates and their prevalence in urinary tract infection. In MDR isolates, we have examined that *E. coli* alone account for more than 59% and rest are Klebsiella and Enterobacter

S. No.	MDR isolates	Percentage (%)
1.	*E coli*	59
2.	*Klebsiella*	10
3.	*Enterobacter*	4

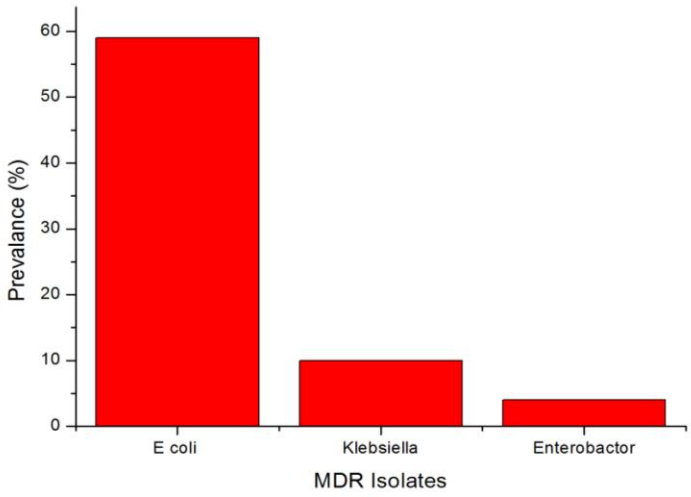

Figure 3: Prevalence of MRR Isolated from UTIs urine samples. The study concludes *E. coli* acquired MDR with the capacity of ESBL producing

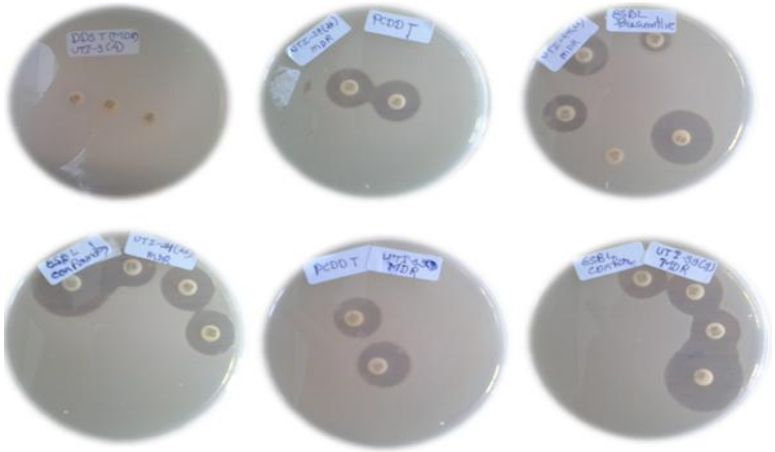

Figure 4: Test for ESBL confirmation, selected urine isolates were subjected to ESBL test using both presumptive, confirmatory (manual) and PCDDT

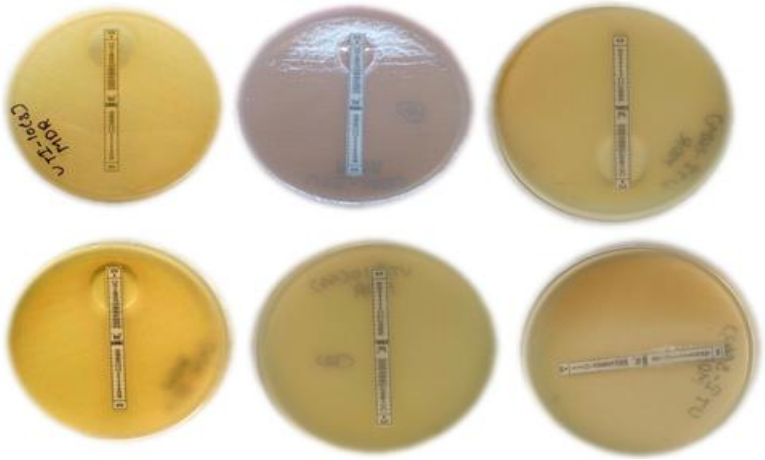

Figure 5: Test for ESBL confirmation, selected urine isolates were subjected to ESBL test using readymade strips

Discussion

We have followed as per Clinical and Laboratory Standards Institute (CLSI) issued recommendations for ESBL screening, for the confirmation of the isolates of Escherichia coli and Klebsiella spp., and for reporting the confirmed organisms. ESBL producing bacteria emerged as major health concern worldwide[18]. To detect ESBL producing bacteria PCDDT test implemented with DDST and it was found to be an inexpensive alternative for the DDST. In our study, we have observed most of the urine bacterial isolates were resistant for NX, NT and CIP significantly. In our study, among 500 selected urine samples from UTIs suspected patients in the City of Bhopal (both Kasturba and Parul hospital), we have reported growth in 298 samples while rest were found negative. The 298 samples were subjected to antibiotic resistance analysis and among those samples 100 reported MDR. Among 100 MDR, 40 isolates (strict MDR based on antibiotic resistance pattern) were further subjected to ESBL test. The ESBL testing was carried out in two ways one using recommended strip and second by ESBL presumptive, DDST and PCDDT. Shukla et al., 2004 have reported similar findings with a higher probability of Klebsiella species in their findings[19].

In the same year, Supriya *et. al.* reported *E. coli* and *Klebsiella* species in their study as major uropathogen[20]. However, the antibiotics sensitivity pattern and MDR pattern was significantly different from current study. Xiong et al. 2002, concludes in their finding prevalence of ESBL producing *E coli* and *Klebsiella species*[21]. Similarly, Lucet et al., 1999 have reported Enterobacter as a major uropathogen in their study capable of ESBL phenotype[22]. All these findings clearly suggest ESBL phenotype in uropathogen is ubiquitous and emerged as a major health concern worldwide. Geographical distribution and ESBL phenotype in different uropathogen relate a natural section

acquiring the capacity to develop drug resistance. In the Southern part of India including Andhra Pradesh, Tamilnadu and Karnataka there has been increasing cases reported for UTIs and ESBL clinical isolates. Subha *et. al.,* 2002 have reported uropathogens primarily *E. coli* and *Klebsiella* in Chennai with ESBL phenotype capable of acquiring resistance against third generation cephalosporin[23]. Cefpodoxime and ceftazidime have been proposed as the indicators of ESBL production as compared to cefotaxime and ceftriaxone[24]. We have successfully reported a high prevalence of ESBL producing uropathogen primarily *E. coli* and Klebsiella species in UTIs patients in City of Bhopal, Madhya Pradesh, India.

Conclusion

The present study highlights the prevalence of ESBL-producing E*. coli*, *Klebsiella pneumoniae* and Enterobacter in Kasturba and Parul Hospital, Bhopal, Madhya Pradesh, India having a significant percentage. The current finding provides a scientific basis for ESBL testing as per recommended guidelines essentially as per Clinical Laboratory Standard Institute guidelines. We have reported 2% of ESBL producing uropathogens mainly *E. coli* in Bhopal population which signifies prevalence of drug resistance in uropathogens. The finding will be useful in finding selective and effective therapy for MDR and ESBL producing not only uropathogens but also other bacteria. The studies will also enlight in finding new measures regular antimicrobial susceptibility surveillance. The finding provides an outline for rational use of antimicrobials and minimizes the emergence of multiple drug resistance.

Acknowledgment

The author would like to thank Department of Microbiology, Department of Microbiology, Barkatullah University, Habib Ganj, Bhopal, Bhopal-462026, Madhya Pradesh, India for providing a facility for current study. Author happily acknowledges Kasturba and Parul Hospital Bhopal, Madhya Pradesh, India for proving urine samples for current study.

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