

## Detection of Extended Spectrum B-Lactamases in Urinary Isolates of Escherichia Coli and Klebsiella Species and Their Antibiogram.

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### Abstract

#### INTRODUCTION

The incidence of extended spectrum  $\beta$ -lactamases (ESBL) producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options. Microorganisms responsible for urinary tract infection such as *Escherichia coli* and *Klebsiella* species have the ability to produce ESBLs in large quantities. There is not enough data on the prevalence of ESBL producers in urinary tract infection in Jharkhand, India. Hence, the present study was undertaken.

#### AIMS AND OBJECTIVES

To detect prevalence of ESBL producers in urinary isolates of *Escherichia coli* and *Klebsiella* species and their antibiograms.

#### MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, RIMS, Ranchi for a period of 6 months (June 2018 to November 2018). A total of 1716 urine samples were processed from patients clinically suspected to have UTI. All *Escherichia coli* and *Klebsiella* species isolated in significant numbers were included in the study. ESBL screening was carried out by using the Kirby-Bauer disk diffusion technique and ESBL confirmation was done by using combination disc method, as per CLSI 2018 guidelines.

#### RESULTS

Of the 1716 urine samples processed 368(21.44%) samples yielded various bacterial isolates. There were 124 *Escherichia coli* and 78 *Klebsiella* spp. among them. ESBL production was observed in 66.93 % (83/124) of *Escherichia coli* and 69.23 % (54/78) of *Klebsiella* species. Resistance to multiple classes of antibiotics was observed among ESBL producers.

#### DISCUSSION AND CONCLUSION

ESBL production has been observed in large percentage of urinary isolates. Patients infected with these strains cannot be treated with  $\beta$ -lactam antibiotics and monobactams. Since Co-resistance to non  $\beta$ -lactam antibiotic like norfloxacin, co-trimoxazole and gentamicin was observed more with ESBL producers, amikacin and nitrofurantoin are found to be alternatives for treating such patients at low cost.

**Keywords:** Extended spectrum  $\beta$ -lactamase (ESBL), *Klebsiella* species, *Escherichia coli*, Antibiogram.

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Date of Submission: 12-05-2020

Date of Acceptance: 24-05-2020

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### I. Introduction

Extended spectrum  $\beta$ -lactamase (ESBL) production confers resistance to all the beta-lactam antibiotics, except carbapenems and cephamycins. In addition, ESBL encoding plasmids also carry genes which encode resistance to other class of antibiotics such as fluoroquinolones, aminoglycosides and sulfonamides.<sup>[1]</sup> ESBLs are plasmid mediated, TEM- and SHV-derived enzymes, first isolated in Western Europe in mid-1980s, most commonly in *Klebsiella* species, followed by *E. coli*.<sup>[2]</sup>

Extended-spectrum  $\beta$ -lactamases (ESBLs) have become increasingly common worldwide and have emerged as a major source of antimicrobial resistance in gram-negative pathogens.<sup>[3]</sup>

Urinary tract infection (UTI) is one of the most common bacterial infections encountered as both hospital-acquired infections and community-acquired infections.<sup>[4]</sup> Microorganisms responsible for urinary tract infection (UTI) such as *E. coli* and *Klebsiella* species have the ability to produce ESBLs in large quantities.<sup>[5]</sup> Complication in UTIs have increased because of the prevalence of extended spectrum  $\beta$ -lactamases (ESBL) producing bacterial pathogens which are also causing many management and epidemiological issues<sup>[1]</sup>

Prevalence of ESBLs varies from institute to institute. Hence the present study was done to know the prevalence of ESBL producers among urinary isolates of *Escherichia coli* and *Klebsiella spp.* and their antibiograms.

## II. Aims And Objectives

To detect prevalence of ESBL producers among urinary isolates of *Escherichia coli* and *Klebsiella species* and their antibiograms.

## III. Materials And Methods

A cross sectional study was carried out in the Department of Microbiology, RIMS, Ranchi for a period of 6 months (June to November 2018). A total of 1716 urine samples from patients clinically suspected to have UTI were processed. Culture was done by the calibrated loop technique, delivering 0.001 ml of urine and plated on Cysteine-Lactose-Electrolyte Deficient (CLED) agar plates. The isolates were identified based on colony morphology, gram's staining and biochemical characteristics. Antimicrobial susceptibility was determined by using Kirby-Bauer disk diffusion technique.

Antimicrobial agent used in this study were Amikacin(30µg), Gentamicin(10µg), Ciprofloxacin(5µg), Nalidixic acid(30µg), Norfloxacin(10µg), Nitrofurantoin(300units), Co-trimoxazole(25µg), Ceftazidime(30µg), Cefotaxime(30µg) and Imipenem (10µg).

ESBL Screening was done based on zone of inhibition produced by ceftazidime(30µg) and cefotaxime(30µg) as per CLSI 2018 guidelines.<sup>[6]</sup>, isolates showing zone of inhibition  $\leq 22$  mm for Ceftazidime and  $\leq 27$  mm for Cefotaxime by disk diffusion method were considered potential ESBL producers.

ESBL Confirmation was done by using combined disk method, according to the Clinical Laboratory Standards Institute (CLSI) Guidelines 2018<sup>[6]</sup>. Ceftazidime (30 µg) and Cefotaxime (30 µg) disks alone and their combinations with clavulanic acid (30 µg/10 µg) were used. A  $\geq 5$  mm increase in zone of inhibition diameters for either of cephalosporin disks or their respective cephalosporin/clavulanate disks was interpreted as ESBL producer. *K.pneumoniae* ATCC 700603(positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control for ESBL tests.

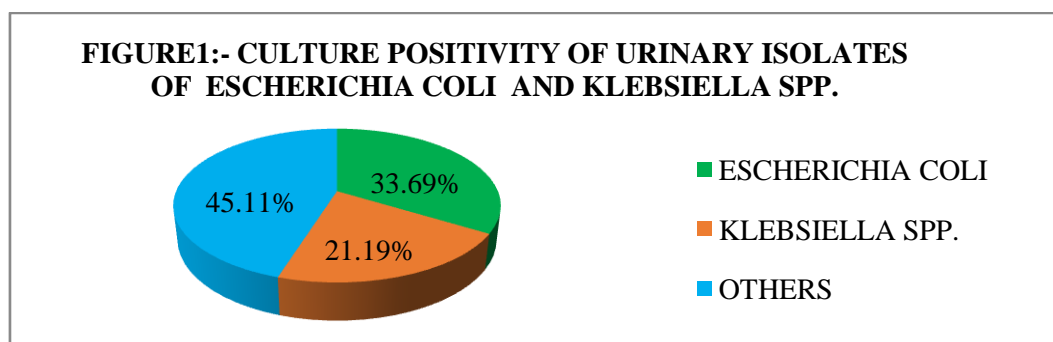
## IV. Results

Of the 1716 urine samples processed, 368 (21.44%) samples yielded various bacterial isolates. Among them 124 (33.69%) were *Escherichia coli* and 78 (21.19%) were *Klebsiella species* (figure 1). ESBL production was observed in 66.94 % (83/124) of *Escherichia coli* and 69.23 % (54/78) of *Klebsiella species* (table 1). These ESBL isolates were obtained from 52 (37.96%) male and 85(62.04%) female (table 2). Females showed a higher rate of isolation of ESBL producing *E.coli* 61.45% (n=51/83) and *Klebsiella species* 62.96 % (n=34/54)(table 3). Higher occurrence of ESBL producing uropathogens seen in the adult age group of 16–45 years (table 4). Only 26 (18.98 %) ESBL-producing isolates were from outpatients and majority 81.02% of ESBL producing isolates were from inpatients.

The overall antimicrobial resistance patterns of the isolates against different antibiotics shown in table 5. Comparison of overall antimicrobial resistance patterns of the ESBL producing and non ESBL producing isolates against different antibiotics shown in table 6.

The antibiogram of ESBL and Non- ESBL producer *Escherichia coli* shown in figure-2.

The antibiogram of ESBL and Non- ESBL producer *Klebsiella species* shown in figure-3.



**Table 1:** Distribution of ESBL and NON –ESBL producing isolates:-

Isolates	ESBL isolates		NON-ESBL isolates		Total	
	N	%	N	%	N	%
E.coli	83	66.94%	41	33.06%	124	61.32%
Klebsiella spp.	54	69.23%	24	30.77%	78	38.61%

Total	137	67.82%	65	32.18%	202	100%
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**Table 2:** Gender wise distribution of overall ESBL and NON-ESBL producer isolates:-

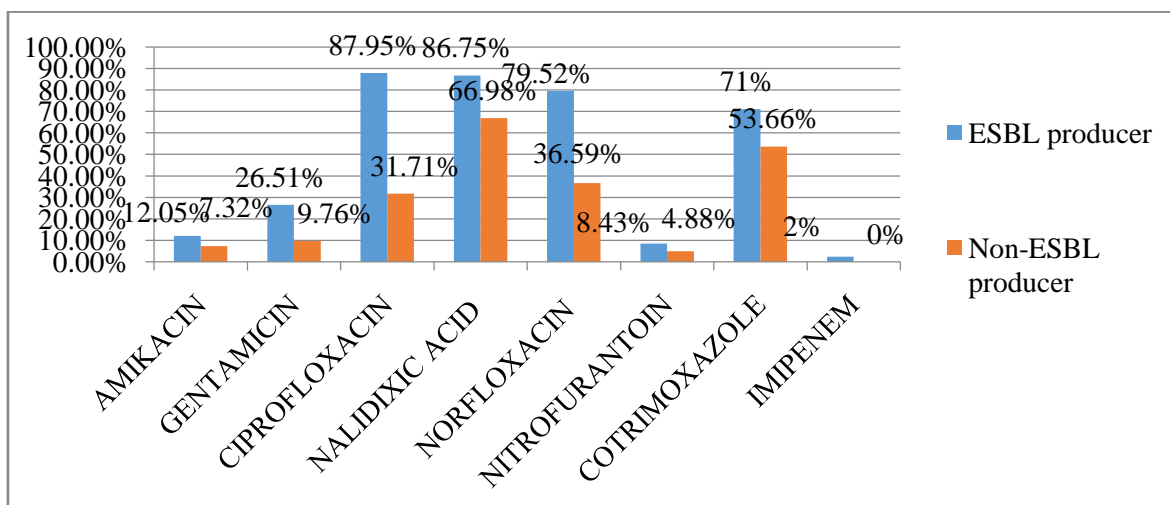
Gender	ESBL isolates		NON-ESBL isolates		Total	
	N	%	N	%	N	%
Male	52	37.96%	27	41.54%	79	39.11%
Female	85	62.04%	38	58.46%	123	60.89%
Total	137	100%	65	100%	202	100%

**Table 3:** Gender wise distribution of ESBL producing isolates among E.coli and Klebsiella species:-

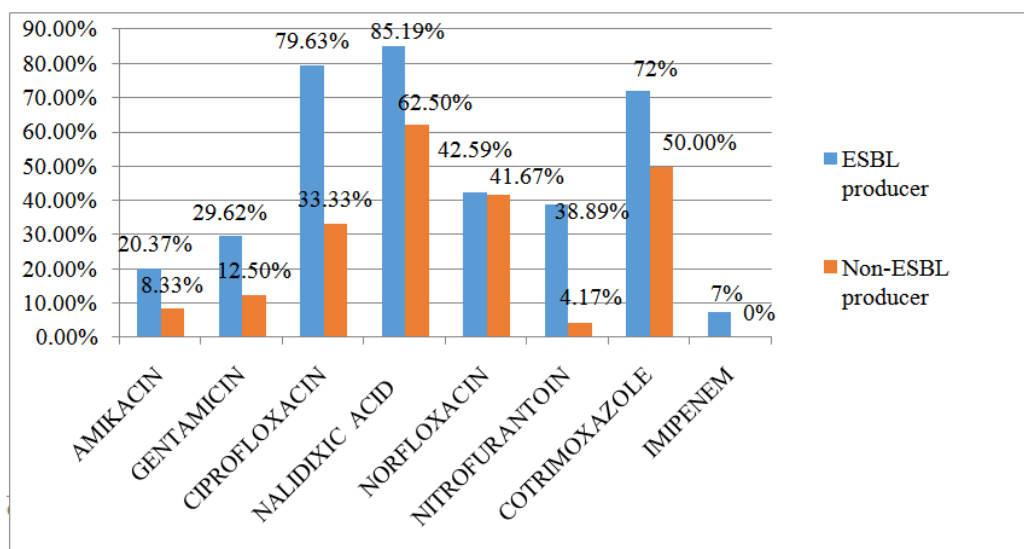
Gender	ESBL producing E.coli		ESBL producing Klebsiella spp.		Total	
	N	%	N	%	N	%
Male	32	38.55%	20	37.04 %	52	37.96%
Female	51	61.45%	34	62.96%	85	62.04%
Total	83	100%	54	100%	137	100%

**Table 4:** Age wise distribution of overall ESBL and NON-ESBL producing isolates:-

Age in years	ESBL producer	Non-ESBL producer	Total
0-15	6	9	15
16-30	60	14	74
31-45	47	18	65
46-60	14	7	21
>60	10	17	27
Total	137	65	202



**Figure 2:** Resistance Pattern of Escherichia coli in relation to ESBL production.



**Figure 3:-** Resistance Pattern of Klebsiella species in relation to ESBL production

**Table 5:** Overall antimicrobial resistance patterns of the isolates against different antibiotics

Antibiotics	Isolates n= 202 (124E.coli + 78 Klebsiella spp.)	
	N	%
Amikacin	26	12.87
Gentamicin	45	22.28
Ciprofloxacin	137	67.82
Nalidixic acid	158	78.23
Norfloxacin	114	56.44
Nitrofurantoin	31	15.35
Cotrimoxazole	132	65.35
Cefotaxime	137	67.82
Ceftazidime	137	67.82
Imipenem	6	2.97

**Table 6:** Comparison of overall antimicrobial resistance patterns of the ESBL and Non-ESBL isolates

Antibiotics	ESBL(n= 137/83+54)		NON-ESBL(n= 65 /41+24)	
	N	%	N	%
Amikacin	21	15.33	5	7.70
Gentamicin	38	27.74	7	10.77
Ciprofloxacin	116	84.67	21	32.31
Nalidixic acid	118	86.13	40	61.54
Norfloxacin	88	64.23	25	38.46
Nitrofurantoin	30	21.90	3	4.61
Cotrimoxazole	98	71.53	34	52.31
Imipenem	6	4.38	0	0

## V. Discussion

Extended spectrum  $\beta$ -lactamases (ESBLs) have become a widespread serious problem. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients.<sup>[7]</sup> The continued emergence of ESBLs presents therapeutic challenges to the clinician because emergence of ESBLs reduces therapeutic options.<sup>[8]</sup>

The present study highlights the prevalence of ESBL producing urinary isolates of Escherichia coli and Klebsiella species in area of Jharkhand, India. It is worrisome that the present study reports an alarmingly high prevalence of ESBL producing strains among urinary isolates of Escherichia coli and Klebsiella species. The prevalence of ESBL producing Escherichia coli is 66.94% and Klebsiella species is 69.23 %. This is higher than that reported in a study carried out by Babypadmini S et al.<sup>[5]</sup>, BC Metri et al<sup>[9]</sup> Taneja et al<sup>[10]</sup> and Tankhiwale SS et al<sup>[11]</sup>.

Babypadmini S et al had observed ESBL production in 41% of E.coli (143/353) and 40% of Klebsiella pneumoniae (23/58),<sup>[5]</sup> BC Metri et al had observed ESBL production in 40.4 % ( 69/171) of E. coli and 44.9% (26/58) of K.pneumoniae<sup>[9]</sup>, Taneja et al had reported 40.2% E. coli isolates and 51.2% of K. pneumoniae to be ESBL producers<sup>[10]</sup> and in a study conducted in 2003 by Tankhiwale SS et al only 18.5% E.coli isolates and 25.6% of K.pneumoniae isolates was ESBL producer<sup>[11]</sup> which was comparatively lower than that reported in the present study.

This variation in prevalence of ESBL producing strains might be because of several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital.

ESBL production was more prevalent in K.pneumoniae strains as compared to that in Escherichia coli strains, which agrees with findings reported in other studies (carried out by Meeta S et al, BC Metri et al, Tankhiwale SS et al and Anil Chander et al.)<sup>[7, 9, 11, 12]</sup>

Females showed a higher rate of isolation of ESBL producing E.coli 61.45% (n=51/83) and Klebsiella species 62.96 % (n=34/54), which parallels the findings as reported earlier.<sup>[13]</sup> This study revealed a higher occurrence of ESBL producing uropathogens in the adult age group of 16–45 years, which is similar to that reported in a study done in Pakistan.<sup>[14]</sup> and majority of ESBL producing isolates were from inpatients (81.02%).

A very high MDR rates of 87.95% (n=73/83) and 83.33 % (n=45/54) among ESBL positive E.coli and Klebsiella species were obtained in this study. A comparable MDR rate of 91.66% among E.coli and 87.5% among K.pneumoniae isolates was reported in a study done in Nepal.<sup>[12]</sup>

In present study none of the strains of ESBL positive E.coli and Klebsiella species were found to be sensitive to all the antimicrobial agents tested.

In present study resistance rate to cotrimoxazole was 71.08 % among ESBL producing strain of E.coli and 72.22% among ESBL producing strain of Klebsiella species. A comparable resistance rate of 85% and 58.33% to co-trimoxazole was observed among ESBL producing E.coli and K.pneumoniae isolates in a study conducted by Anil Chander et al.<sup>[12]</sup> A striking finding in the present study was that the quinolones, norfloxacin and nalidixic acid demonstrated a high resistance 79.52% and 86.75% among ESBL positive E.coli, while 42.59 % and 85.19 % among ESBL positive Klebsiella species. This parallels with the findings of the studies done in Indore, India<sup>[15]</sup> and Bangladesh.<sup>[16]</sup>

A lower resistance rates varying from 24% to 44% to norfloxacin had been reported in European countries.<sup>[17]</sup> This probably reflects a better management of these clinically significant infections in resource-rich countries.

In the present study resistance rate to the aminoglycosides, gentamicin and amikacin was 29.63% and 20.37% among ESBL producing strain of Klebsiella species and 26.51% and 12.05 % among ESBL producing strain of E.coli respectively. In contrast in a study done in Indore, India by Pathak A et al K. pneumoniae showed resistance to gentamicin 69% and amikacin 38% , while 59% and 33% resistance rates were shown by E.coli isolates which is higher than that reported in present study.<sup>[15]</sup> A resistance rate of 46.7% to gentamicin was shown by K.pneumoniae isolates in a report from Karachi, Pakistan<sup>[18]</sup> which is also higher than that reported in present study.

The resistance rates of ESBL positive E.coli to nitrofurantoin were 8.43% .Parallel resistance rate of 12% by E.coli isolates was shown in a report from Indore, India.<sup>[15]</sup>

A significant finding in this study was that aminoglycosides and nitrofurantoin proved to be the optimal drugs. This may be due to the restricted use of these drugs in our hospital setting and nitrofurantoin is usually reserved to be prescribed only in cases of UTIs since it is excreted and concentrated in urine.

Most of the ESBL positive E.coli and Klebsiella species strains in this study were sensitive to imipenem 97.59% and 92.59% respectively.

Multi-center studies involving major health-care facilities in other parts of the country are required to have a more clear picture of ESBL producing uropathogens. Further, molecular epidemiological studies of resistance genes among the uropathogens would provide us much needed details on bacterial clones circulating in this region.

## VI. Conclusion

Urinary tract infection is one of the most frequent conditions in medical practice affecting people of all ages. This report documents the emergence and occurrence of ESBL producing E.coli and Klebsiella spp. in urinary isolates in Jharkhand, India. A high prevalence of ESBL producing E.coli (66.94%) and Klebsiella species (69.23%) was observed at our institute. The majority of ESBL producing isolates were resistant to the antibiotics commonly used for treatment of UTI. Imipenem was the most effective antibiotic and could be the drug of choice for treatment of infections caused by ESBL strains.

The increasing levels of ESBLs may be attributed to the increased empirical administration of third generation cephalosporins. This reduces the clinical utility of cephalosporins. In order to preserve these effective antimicrobials, physicians must rely on routine urine culture and sensitivity tests and treat the patients accordingly.

## Reference

- [1]. Elsayed TI, Ismail HA, Elgamal SA et al. The occurrence of multidrug Resistant E. coli which produce ESBL and Cause Urinary Tract Infections. J Appl Microbial Biochem. Vol. 1 No.2:8.
- [2]. Chaudhary U, Aggarwal R. Extended spectrum  $\beta$ -lactamases (ESBL) – An emerging threat to clinical therapeutics Indian J Med Microbiol 2004;22:75-80.
- [3]. S. Thenmozhil, K. Moorthy, B.T. Suresh kumar, M. Suresh, Antibiotic Resistance Mechanism of ESBL. Producing Enterobactriaceae in Clinical Field. A Review.
- [4]. Nandagopal B, Sankar S, Sagadevan k, Arumugam H, Jesudason M V, Aswathaman K, Nair A. Frequency of extended spectrum  $\beta$ -lactamase producing urinary isolates of Gram-negative bacilli among patients seen in a multispecialty hospital in Vellore district, India. Indian J Med Microbiol 2015;33:282-285.
- [5]. Babypadmini S, Appalaraju B, Extended spectrum  $\beta$ -lactamases in a tertiary care hospital Indian J Med Microbiol 2004;22:172-174.
- [6]. Clinical Laboratory Standard Institute Performance standards for antimicrobial disc susceptibility tests, 28<sup>th</sup> ed. CLSI supplement M100. Wayne PA : Clinical Laboratory Standard Institute; 2018.
- [7]. Meeta Sharma, Sati Pathak, and Preeti Srivastava. Prevalence and antibiogram of Extended Spectrum  $\beta$ -Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. J Clin Diagn Res. 2013 Oct; 7(10): 2173-2177. Published online 2013 Oct 5. Doi:10.7860/JCDR/2013/6460.3462
- [8]. Farzana R, Shamsuzzaman SM, Mamun KZ, Shear P. Antimicrobial susceptibility pattern of extended spectrum  $\beta$ -lactamase producing gram-negative bacteria isolated from wound and urine in a tertiary care hospital Dhaka city, Bangladesh, Southeast Asian J Trop Med Public Health 44:96-103.
- [9]. BC Metri, P Jyothi, BV Peerapur. Detection of ESBL in Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infection. Indian J Nephrol, 22(5), 2012, 401-402.

- [10]. Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL and Amp-C  $\beta$ -lactamases and susceptibility to newer antimicrobial agents in complicated UTI. *Indian J Med Res.* 2008; 127:85-8.
- [11]. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J Med Res.* 2004;120:553-6.
- [12]. Anil Chander and Chandrika Devi Shrestha Prevalence of extended spectrum  $\beta$ -lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal.
- [13]. Ahmed SM, Jakribettu RP, Koyakutty S, Arya B Shakir VPA. Urinary tract infections – an overview on the prevalence and the anti-biogram of gram negative uropathogens in a tertiary care center in North Kerala, India. *J Clin Diagn Res.* 2012;6:1192-1195.
- [14]. Ullah F, Malik SA, Ahmed J, Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. *Afr J Biotechnol.* 2009;8(16):3921-3926.
- [15]. Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R, Lundborg CS High prevalence of extended spectrum  $\beta$ -lactamase producing pathogens; results of a surveillance study in two hospitals in Ujjain, India. *Infect Drug Resist.* 2012;5: 65-73.
- [16]. Alipourfard I, Nili NY. Antibiogram of extended spectrum  $\beta$ -lactamase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital samples. *Bangladesh J Med Microbiol.* 2010;4(1):32-36.
- [17]. Van der Donk CFM, van de Bovenkamp JHB, De Brauwier EIGB, De Mol P, Feldhoff K-H, Kalka-Moll WM. Et al Antimicrobial resistance and spread of multi drug resistant *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine. *PLoS One.* 2012;7(10):e47707. Doi:10.1371/journal.pone.0047707.
- [18]. Abdullah FE, Mushtaq A, Irshad M Rauf H, Afzal N, Rasheed A. Current efficacy of antibiotics against *Klebsiella* isolates from urine samples – a multi-centric experience in Karachi. *Pak J Pharm Sci.* 2013; 26(1):11-15. [PubMed]

Manoj Kumar, et. al. "Detection of Extended Spectrum B-Lactamases in Urinary Isolates of *Escherichia Coli* and *Klebsiella* Species and Their Antibigram." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(5), 2020, pp. 01-06.