

Original Article

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESBL PRODUCING GRAM NEGATIVE BACILLI IN 200 CASES OF URINARY TRACT INFECTIONS

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ABSTRACT

**Objective:** Isolation and identification of Extended Spectrum Beta Lactamases from urinary isolates and to study their drug sensitivity pattern.

**Methods:** The study was done on 200 patients with suspected urinary tract infection from Rajindra Hospital, Patiala. Urine samples were processed for significant bacteriuria. The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as per the CLSI recommendations. ESBL production was tested by using the double-disk approximation test and the combination disk method.

**Results:** Out of the total of 200 urine samples, there were 84 (42%) culture positive cases, out of which 78 (92.9%) were found to be gram negative bacilli. E. coli was found to be the commonest uropathogen (60.3%). The percentage of ESBL producers was 69.2%. Maximum number of ESBLs were found among E. coli isolates i.e. 80.9%. E. coli was found to be sensitive to imipenem (97.9%) followed by nitrofurantoin (91.5%), amikacin (76.6%), piperacillin-tazobactam (68%) and gentamicin (53.2%). It was relatively resistant to ampicillin. Ciprofloxacin showed sensitivity of 29.8% in E. coli.

**Conclusion:** E. coli and other isolates were more sensitive to impaired. Nitrofurantoin and piperacillin-tazobactam compared to the other antibiotics tested and therefore these may be the drugs of choice for treatment of infections that are caused by ESBLs.

**Keywords:** Urinary tract infection, Extended-spectrum  $\beta$ -lactamases, Resistance.

INTRODUCTION

Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the twentieth century. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem.

There is one group of  $\beta$ -lactamases that is occasionally found in certain species of gram negative bacilli, usually *Klebsiella pneumoniae* and *Escherichia coli*. These enzymes are termed extended-spectrum  $\beta$ -lactamases (ESBLs) because they confer upon the bacteria the additional ability to hydrolyze the  $\beta$ -lactam rings of cefotaxime, ceftazidime or aztreonam [1]. Microorganisms responsible for urinary tract infection (UTI) such as *E. coli* and *Klebsiella* spp. Have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat [2].

MATERIALS AND METHODS

The study was conducted on 200 urine samples received from clinically suspected patients of lower urinary tract infections coming from Rajindra hospital, Patiala. The samples were processed for significant bacteriuria in the department of Microbiology, Government Medical College, Patiala. Samples were tested for ESBL production by using the double-disk approximation test and the combination disk method which are recommended by CLSI.

Antibiotic sensitivity testing

The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as per the CLSI recommendations by using various antibiotics (in  $\mu\text{g}$ ) viz., ceftazidime(30), Cefepime(30), Cotrimoxazole (25), amikacin (30), gentamicin(10), ciprofloxacin (5), piperacillin-tazobactam (100/10), nitrofurantoin(300) and Imipenem (10). *K. Pneumoniae* ATCC 700603 was used as a control strain for positive ESBL production and *E. coli* 25922 was used as a negative control for ESBL production.

Double-disk approximation test

A lawn culture of test strain on Mueller Hinton Agar (Hi-Media, Mumbai) was exposed to a disc of later generation cephalosporin, eg. Cefotaxime (30 $\mu\text{g}$ ) or ceftazidime (30 $\mu\text{g}$ ) and a disc of amoxiclav (20 $\mu\text{g}$  amoxicillin/10 $\mu\text{g}$  clavulanic acid) was arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone produced by the later generation cephalosporin tested on its own. After overnight incubation, if the test strain had an extended spectrum  $\beta$ -lactamase, the inhibition zone around the cephalosporin disc was extended on the side nearest to the co-amoxiclav disc [3].

Combination disk method

In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated on the surface of a Mueller Hinton Agar plate. The cefotaxime (30 $\mu\text{g}$ ) and cefotaxime-clavulanic acid (30 $\mu\text{g}$ / 10 $\mu\text{g}$ ) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 $\mu\text{g}$ ) and ceftazidime-clavulanic acid (30 $\mu\text{g}$ / 10 $\mu\text{g}$ ) disks were placed 20 mm apart. After incubating overnight at 37 $^{\circ}\text{C}$ , a  $\geq$  5-mm increase in the zone diameter for either antimicrobial agent which was tested in combination with clavulanic acid vs. Its zone when tested alone, was interpreted as positive for ESBL production [4].

Table 1: Percentage of various pathogens in culture positive cases

Organisms	No. of cases	Percentage
<i>E. coli</i>	47	60.3%
<i>Klebsiella pneumoniae</i>	16	20.5%
<i>P. aeruginosa</i>	7	9%
<i>Proteus</i> spp.	6	7.7%
<i>Enterobacter</i>	2	2.5%
Total	78	100%

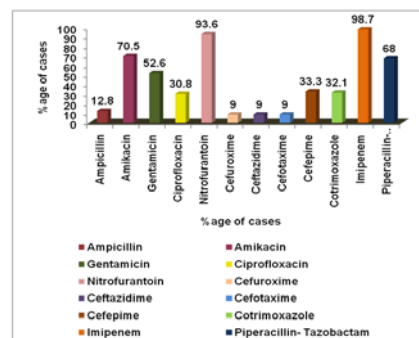
## RESULTS

Out of total 200 urine samples, there were 84 (42%) culture positive cases, out of which 78 (92.9%) were found to be gram negative bacilli. *E. coli* was found to be the commonest uropathogen (60.3%) as shown in Table 1.

*E. coli* was found to be sensitive to imipenem (97.9%) followed by nitrofurantoin (91.5%), amikacin (76.6%), piperacillin-tazobactam (68%) and gentamicin (53.2%). It was relatively resistant to ampicillin. *Klebsiella pneumoniae* was also found to be sensitive to imipenem (100%) followed by nitrofurantoin (93.8%), amikacin (75%), piperacillin-tazobactam (62.5%) and gentamicin (56.3%). *Pseudomonas aeruginosa*, *Proteus* spp.

And *Enterobacter* showed 100% sensitivity to imipenem and nitrofurantoin. Ciprofloxacin showed sensitivity of 29.8% in *E. coli*, 25% in *Klebsiella pneumoniae*, 42.9% in *Pseudomonas aeruginosa*, 33.3% in *Proteus* spp. and 50% in *Enterobacter* as shown in Figure 1.

The percentage of ESBL producers was 69.2%. Maximum ESBL producers were found among *E. coli* isolates i.e. 80.9% followed by *Klebsiella pneumoniae* (75%).



**Fig. 1: Susceptibility pattern showing percentage of different bacterial isolates sensitive to various antimicrobial agents.**

*Pseudomonas aeruginosa*, *Proteus* spp. And *Enterobacter* were 14.3%, 33.3% and 50% respectively as shown in Table 2. Double disk approximation test was less sensitive (74.07%) in detecting the ESBLs than the Combination disc method (100%).

**Table 2: Distribution of ESBL producers among different gram negative isolates**

Organism	Double disk approximation test		Combination disc method		Chi square
	No. of cases	%age	No. of cases	%age	
<i>E. coli</i>	30	63.8%	38	80.9%	1.68838 (p<0.9)
<i>K. pneumoniae</i>	9	56.3%	12	75.0%	
<i>P. aeruginosa</i>	0	0%	1	14.3%	
<i>Proteus</i> spp.	1	16.7%	2	33.3%	
<i>Enterobacter</i>	0	0%	1	50%	

## DISCUSSION

*E. coli* (60.3%) was found to be the commonest organism responsible for urinary tract infections. Manjunath et al. showed 66.8% isolation of *E. coli* [5]. *E. coli* was found to be sensitive to imipenem (97.9%) followed by nitrofurantoin (91.5%), amikacin (76.6%) and piperacillin-tazobactam (68%). Babypadmini et al. showed the susceptibility of ESBL producers to imipenem, nitrofurantoin and amikacin to be 100%, 89% and 86% respectively [2]. A study done by Umadevi et al showed 100% sensitivity to imipenem, 84% sensitivity to piperacillin-tazobactam and 68% sensitivity to amikacin [6]. The percentage of ESBL producers was 69.2%. A study done by Mathur et al and Umadevi et al. showed 68% and 75% prevalence of ESBL producers respectively [6,7].

## CONCLUSION

Thus it can be concluded that the incidence of ESBL's can be minimized by judicious use of antibiotics and implementation of appropriate infection control measures to control the spread of these strains in the hospital. Moreover, our study concludes that *E. coli* and other isolates were more sensitive to imipenem, nitrofurantoin and piperacillin-tazobactam compared to the other antibiotics tested and therefore these may be the drugs of choice for treatment of infections that are caused by ESBLs.

## CONFLICT OF INTERESTS

Declared None

## REFERENCES

- Jawetz, Melnick, Adelberg. Antimicrobial Chemotherapy. Medical Microbiology, 25<sup>th</sup> ed. Lange Publishers; 2010. p. 339-41.
- Babypadmini S, Appalaraju B. Extended-spectrum  $\beta$  Lactamase in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. *Ind J Med Microbio* 2004;22(3):172-4.
- Mackie and McCartney Practical Medical Microbiology. Laboratory control of antimicrobial therapy, 14<sup>th</sup> ed. Churchill Livingstone; 2008. p. 169.
- Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement ed. CLSI document M100-S20. Wayne, PA: CLSI; 2010.
- Manjunath GN, Prakash R, Annam V, Shetty K. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. *Int J Bio Med Res* 2011;2(2):504-7.
- Umadevi S, Kandhakumari J, Joseph NM, Kumar S, Easow JM, Stephen S, et al. Prevalence and antimicrobial susceptibility pattern of ESBL producing gram negative bacilli. *J Clin Diag Res* 2011;5(2):236-9.
- Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. *Ind J Med Res* 2002;115(4):153-7.