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RESEARCH ARTICLE

DETECTION OF EXTENDED SPECTRUM BETA - LACTAMASE PRODUCING *E. COLI* & *KLEBSIELLA SPECIES* CAUSING URINARY TRACT INFECTION

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ABSTRACT

Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated ß-lactamases which confer resistance to broad spectrum beta-lactam antibiotics. The frequency of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of the therapeutic options. This study was done to determine the susceptibility pattern of different antibiotics to ESBL producing *Escherichia coli* and *Klebsiella spp.* isolated from urine samples. Out of 70 urine samples, 62 samples showed bacterial growth. *E.coli* (61.29%) found to be most common bacteria in urinary tract infection followed by *Klebsiella spp.* (45.16%). Antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method. Isolates were highly resistance to Gentamicin followed by Tetracyclin, Ampicillin and Amikacin. Gentamicin showed resistance against *E.coli* and *Klebsiella spp* were 89.47% and 85.71% respectively. While Tetracyclin and Ampicillin showed 80% and 78% resistance to *E.coli and Klebsiella spp.* The isolates were highly susceptible against imipenem and least susceptible to Ciprofloxacin and Norfloxacin against *E.coli* and *Klebsiella spp.* These antibiotics are considered as appropriate antimicrobials for empirical treatment of urinary tract infections. Most of the ESBL producing isolates were multidrug resistant. Continuous monitoring of ESBL production and antimicrobial susceptibility testing are necessary for the treatment of UTI.

KEYWORDS: ESBL, Antibacterial susceptibility pattern, Urinary Tract infection.

INTRODUCTION:

Despite the widespread availability of antibiotics, urinary tract infection (UTI) remains the most common bacterial infection in the human population. Antibiotics are usually given empirically before the laboratory results of urine culture are available [1]. Resistance to antibiotic treatment in patients with urinary tract infections (UTIs) is a representative example of the increasing problem of antimicrobial resistance [2]. Extended spectrum beta lactamases (ESBLs) are the enzymes that mediate resistance to extended spectrum (third generation) cephalosporins and monobactams but do not affect cephamycins or carbapenems. ESBLs pose a major threat in clinical therapeutics [3]. The first ESBL-producing organism was isolated in Germany in 1983. Thereafter, such organisms were reported in the USA following outbreaks of infections caused by these pathogens. ESBL arise by mutations in genes for common plasmid-mediated beta lactamases that alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta lactamases for oxyimino compounds while simultaneously weakening the overall enzyme efficiency [1]. ESBL producing Gram Negative bacteria are increasingly being associated with hospital infections. They can be found in variety of Enterobacteriaceae species. Majority of ESBL producing strains are *K. pneumoniae, K. oxytoca and E.coli* [4]. Worldwide data shows that there is an increasing resistance among UTI pathogens to conventional drugs. Resistance has emerged even to newer, more potent antimicrobial agents [5]. The aim of this study was to detect ESBL-producing gram negative bacteria isolated from the urine of patients based on their susceptibility to antimicrobial agents.

MATERIALS & METHODS:

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS:

During this study, a total of 70 urine cultures were collected from different hospitals of city. This urine samples were collected in sterile plastic containers. Urines samples were further analyze for the bacterial growth. Bacteria were isolated using standard media, including Eosin Methylene Blue (EMB) Agar, CLED agar, Blood agar and MacConkey agar and specimens were inoculated using standard techniques. Plates were incubated at 37ºC for overnight for growth. Identification of all isolates was done on the basis of routine morphological test such as Gram's Staining and Motility, biochemical tests i.e. fermentation of

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sugars, ability to produce indole, reaction on triple sugar iron (TSI) medium, hemolysis on blood agar, citrate utilization etc. The organisms were maintained at 4°C on agar slants for further process.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

The antibacterial susceptibility was tested by disc diffusion method according the Bauer et. al by using Mueller-Hinton Agar [6]. The twelve antimicrobial agents used in this study were: Ampicillin, Amikacin, Amoxicillin, Ceftriaxone, Co-trimoxazole, Ceftazidime, Ciprofloxacin, Gentamicin, Imipenem, Tetracycline, Norfloxacin and Tobramicin. Antibiotic disc were provided by Hi-Media, Mumbai. The results were interpreted as per Clinical and Laboratory Standard Institute [7].

COMBINATION DISC METHOD FOR ESBL:

Testing for ESBL production was carried out using Mueller Hinton agar plates that were inoculated with standardized inoculum of the isolates compared to 0.5 McFarland standards to form a lawn culture. Separate commercial discs containing Ceftriaxone (30 µg) and ceftazidime (30 µg) with and without clavulanic acid (10 µg) were placed over the lawn culture. An increase in zone size of more than or equal to 5 mm for Ceftriaxone and ceftazidime with and without clavulanic acid was considered to indicate ESBL producing strain as described by Carter *et al*.[8].

RESULT & DISCUSSION:

This study undertaken to detect the ESBL producing bacterial strains isolated from patients diagnosed with UTIs in different local hospitals. A total of 70 urine specimens were collected from patients suspected of having UTI, out of which a total number of 62 showed significant bacterial growth. The commonest bacteria found in our study were *E. coli* (61.29%) and *Klebsiella spp.* (45.16%). Urinary tract infection is one of the commonest bacterial infections. The Enterobacteriaceae are the most frequent pathogens detected, causing 84.3% of UTI [9]. All the bacterial isolates were resistance atleast 5 drugs. The resistance percentage was high against Gentamicin, Amikacin, Ampicillin and Tetracyclin. Arsalan *et al*. (2005) reported 51% and 60% resistance to uncomplicated and complicated strains of *E. coli*, which is correlation with our studies [10]. Ampicillin was resistance to *E.coli* (78.94%) and *Klebsiella spp.* (71.42%) this is due to the continuous use of drug for many years. This study showed the similar results with compared with the study of Sushil Kumar Sahu et. al who reported that these strains were highly resistance to ampicillin [11]. The drug showed the high level of resistance toTetracycline, an emerging in clinical isolates in community. According to a report by Noor *et al*.,(2004) tetracycline resistance was found to be 83.9% to *E. coli* while in our study resistance was found to be 84.21% against *E. coli* and 78.57% against *Klebsiella spp.*[12].[Table & Fig. no. 1].

The resistance was high against aminoglycosides i.e., gentamicin and tobramycin, as compare to previous reports Reham *et al*., [13]. The gentamicin showed resistance to *E.coli* (89.47%) and *Klebsiella spp*. (85.71%), whereas tobramycin showed resistance against *E.coli* and *Klebsiella spp.* were 78.94% and 64.82% respectively. Among cephalosporins, resistance was high to Ceftazidime, Ceftriaxone and Co-trimoxazole i.e., 63.15%, 57.89% and 68.42% respectively against *E. coli.* Ko *et al*. (2008) reported approximately same result against *E.coli.* [14] and Ceftazidime among ESBL producing isolates of *E.coli*. It has been reported that the resistance against ceftraxone is very high i.e. 73% in Iran reported by Mehrgan and Rahbar [15]. Ko *et al*., showed that Imipenem is a carbapenem antibiotic, which is highly active against Enterobactereaceae producing ESBL [14]. This drug is highly beta-lactamase stable and has an unusual property of causing a post antibiotic effect on Gram-negative bacteria. The resistance to imipenem was found to be 26.31% against *E. coli* and 28.57% against *Klebsiella spp.* Other drug which was useful for the treatment urinary tract infection was Ciprofloxacin and Norfloxacin. These drugs showed low level of resistance against the isolates.[Table & Fig No.1]. Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in India [16]. Beta lactamse production has often occurred in parallel with an increase in resistance to aminoglycosides producing highly resistant strains. Shahid et. al. reported in India the prevalence of multidrug-resistant bacterial isolates is quite high in our locality [17].

This high degree of resistance could be explained by the fact that these drugs are easily available in market and people take this drug without doctor's prescription from pharmacy. Self-prescribed and relatively cheaper antibiotics used for all type of infection by patients, quakes and doctors and are often taken in inadequate doses resulting in high degree of resistance. Clinicians must depend on more laboratory guidance which was based on local antibiotic susceptibility pattern of patient. Our study showed that ESBL production was high among uropathogens. The situation is uncontrolled due to multiple drug resistance seen in ESBL producers. Hence, routine

ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI.

Table 1: Antibiotic susceptibility pattern of bacteria isolates.

Sr. No.	Antibiotics	*E. coli* n=38			*Klebsiella* spp. n=28		
		S	R	%R	S	R	%R
1.	Ampicillin	8	30	78.94	8	20	71.42
2.	Amikacin	8	30	78.94	8	20	71.42
3.	Amoxicillin	22	16	42.10	14	14	50
4.	Gentamicin	4	34	89.47	4	24	85.71
5.	Imipenem	28	10	26.31	20	8	28.57
6.	Tobramycin	8	30	78.94	10	18	64.82
7.	Ciprofloxacin	26	12	31.57	18	10	35.71
8.	Norfloxacin	24	14	36.84	18	10	35.71
9.	Tetracyclin	6	32	84.21	6	22	78.57
10.	Ceftazidime	14	24	63.15	17	16	57.14
11.	Co-trimoxazole	12	26	68.42	18	18	64.28
12.	Ceftriaxone	16	22	57.89	18	15	53.57

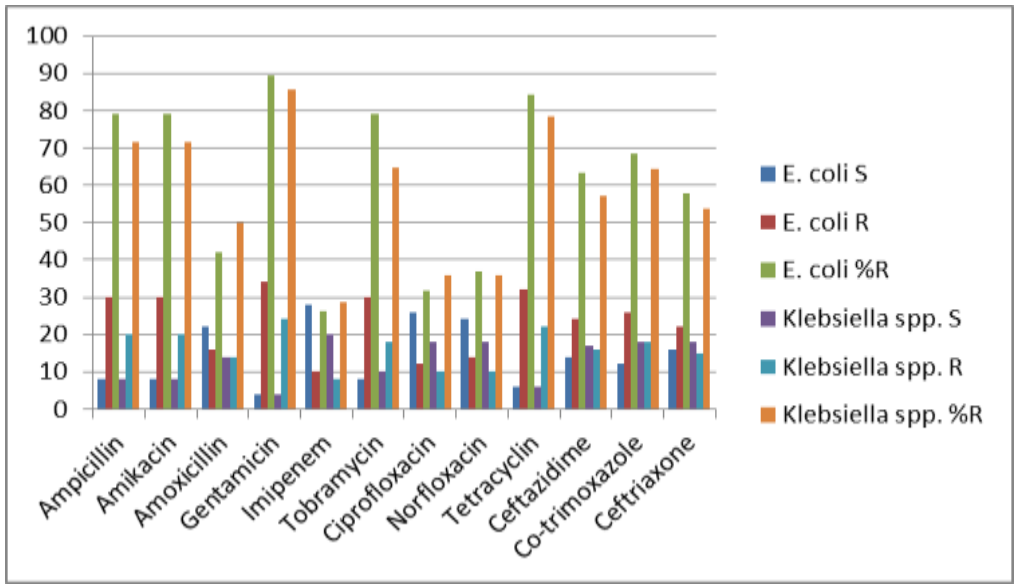

Figure 1: Diagrammatic representation of percentage of Resistance of all isolates.

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