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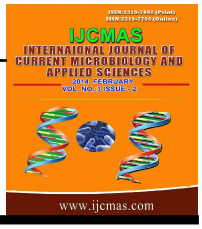

Original Research Article

Detection of ESBL among the Gram negative uropathogens and their antibiotic resistance pattern in a rural medical college hospital North Kerala, India

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K e y w o r d s

Uropathogens; ESBL; urinary tract infections antibiotic resistance pattern.

A B S T R A C T

Despite the advances in diagnostic methods, availability of antimicrobials and awareness among the people, urinary tract infections continue to remain a major health problem, responsible for significant rise in alarming complications especially among the hospital acquired urinary tract infections as most often it is caused by multidrug resistant pathogens. Knowing Resistance pattern of such multidrug resistant pathogens will help in the appropriate usage of anti microbial agents and prevent further emergence of resistant strains. The study was carried to determine the prevalence of ESBL among the aerobic Gram negative uropathogens and their antimicrobial resistance pattern .The urine samples from patients at a medical college hospital, over a period of 6 months from June 2013 to Nov2013, were processed according to standard protocol. The ESBL isolated were subjected to antimicrobial susceptibility tested by modified Kirby-Bauer's disc diffusion method as per the CLSI guidelines. The data interpreted using WHO Net Surveillance Software. Out of the total 1659 urine samples processed 1564 (94.2%), 343 (20.6%) Gram negative isolates were obtained out of that 65(18.95%) were ESBL. sample wise ESBL isolation rate was highest in catheterized urine samples (18.94%) as shown in ESBL prevalence was highest in *citrobacter koseri* (33.3%) followed by *E.coli* (21.28%).Sex wise in females the ESBL isolation was 63%. Age wise ESBL isolation was highest in >50yrs. Among the antibiotics least resistance was shown by imipenem (1.5%) followed by amikacin (10.8%), highest resistance was shown by nalidixic acid (98.5%)..A constant monitoring system should be in place in the microbiology laboratory for monitoring the ESBL not only in urine samples, and knowledge of their antibiotic resistance patterns will be of great help in dealing with such multidrug resistant strains. We have found the WHONET software very helpful tool for this purpose.

Introduction

Urinary tract infection includes the infection of urethra, bladder, ureters, and kidneys, which comprise the urinary tract.

UTI is an important cause of morbidity and mortality in both developing and developed countries of the world, affecting

all age groups and both the sexes(Dogra V et al 2012) (Alipourfard I et al 2010) (Akram M et al 2007). Most UTI usually occur by the ascending route after entry via the urethral meatus, this is by far the most common route of infection in the female and in association with instrumentation, in both sexes(Dash M et al 2013) . Gram negative bacteria are most often implicated in causing UTI (Dogra V et al 2012) (Akram M et al 2007). ESBLS are Gram-negative bacteria that produce an enzyme; beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillins and cephalosporins and render them ineffective for treatment. ESBLs are commonly spread via direct and indirect contact with colonized/infected patients and contaminated environmental surfaces, most commonly spread via unwashed hands of health care provider's .Detection of ESBL producing organisms from urine samples will be useful as this represents an epidemiologic marker of colonization. Hence, the present study was designed to detect ESBL production among uropathogens and knowing their antibiotic susceptibility patterns for preparing the antibiotic policy and for detecting and control of the outbreak of ESBL producing organisms in a hospital is of critical importance

Materials and Methods

This prospective study conducted in Department of Microbiology, in a tertiary care centre in north Kerala after getting the institutional ethical committee approval for a period of 6 months from June 2013 to Nov2013. All Gram negative aerobic bacteria isolated from the mid –stream and catheterized urine sample of patients who were clinically suspected of suffering from UTI as per the standard protocol were identified based on the colony characteristics, Gram's staining and biochemical reactions. Only patients who had significant bacteriuria (>10^5 CFU/mL) were included in the microbiological analysis. All the strains screened out for ESBL production were also subjected to confirmation by using the phenotypic confirmatory disc diffusion test (PCDDT), as recommended by the CLSI (wayne P 2009). ESBL isolates were further subjected to Antibiotic susceptibility testing using the Kirby-Bauer disc diffusion. The following antibiotic discs (drug concentration in µg) were used: amikacin (30), ceftazidime (30), cefotaxime (30), gentamicin (15), imipenem (10), ciprofloxacin (5), nalidixic acid (30), norfloxacin (10) and nitrofurantoin (300)(6). Data was analysed using the WHONET antibiotic resistance surveillance soft ware

Results and Discussion

Out of the total 1659 urine samples processed 1564(94.2%) were clean voided mid stream urine samples and 95(5.72%) were catheterized urine samples. 343(20.6%) Gram negative isolates were obtained from total urine samples processed out of that 65(18.95%) were ESBL, out of this 47(72.3%) from the midstream and 18(27.6%) from the catheterized . sample wise ESBL isolation rate was highest in catheterized urine samples (18.94%) as shown in (Fig.1). Among individual gram negative isolates ESBL prevalence was highest in *Citrobacter koseri* (33.3%) followed by *E.coli* (21.28%) , klebsiella pneumonia (15.9%) as shown in fig 1 overall among all the ESBL isolates highest isolation was in *Escherichia coli* (68%) followed by *Klebsiella pneumoniae* (20%), *Citrobacter freundii* (5%), *Citrobacter koseri* (5%) and

Klebsiella oxytoca (2%) as shown in fig 2. Sex wise in females the ESBL isolation was 63% and in males it was 36.9% as shown in Fig. 3. Age wise among the ESBL isolates it was highest in the age group 51-60yrs (20%) ,71-80yrs (20%) , 81-90yrs (20%) followed by 61-70yrs (18.4%) , 41-50yrs(15.3%) as shown in fig 4.

Among the antibiotics tested by Kirby-Bauer disc diffusion method least resistance was shown by imipenem (1.5%) followed by amikacin (10.8%) and nitrofurantoin (32.3%) , highest resistance was shown by nalidixic acid (98.5%) followed by ciprofloxacin (86.2%), ofloxacin (83.1%), levofloxacin (81.5%) , norfloxacin (81.5%) gentamicin (63.1%) as shown in fig .5 & 6.

Ours is a tertiary care set up with around 600 beds for inpatients, it has all the medical departments including the super speciality departments. ESBL are multi drug resistant and have to be monitored to prevent their spread so we carried out this study mainly to the know prevalence of ESBL in the urine samples received in our microbiology laboratory and also to know susceptibility patterns. 343(20.6%) Gram negative isolates were obtained from total urine samples processed out of that 65(18.95%) were ESBL which was less than reported by (A. K. Borthakur et al 2012) (24.56%), (Ritu Aggarwal et al 2009)(36%)**,** Among the individual gram negative isolates ESBL prevalence was highest in citrobacter koseri (33.3%) followed by E.coli (21.28%) , klebsiella pneumonia (15.9%) as shown in fig 1 it was in correlation with (Sood S et al 2012) (23.83%) E. coli strains and (8.69%) Klebsiella strains lower than (Baby padmini et al2004) E.coli (41%) , klebsiella pneumonia(40%) . Among all the ESBL isolates E.coli was found to predominate (68%) followed by klebsiella pneumonaiae (20%) as shown in fig 2 in correlation to that reported by (Shaifali I et al 2012) (59.1%)(10) (Dash M et al 2013) (68.8%) and(Sood S et al 2012) (62.42%) but less than that reported by (Dogra V et al 2012) (44.1%). sample wise ESBL isolation rate was highest in catheterized urine samples (18.94%) which shows that hospital acquired infections are usually caused by multidrug resistant strains .Age wise there was increased prevalence of ESBL >40yrs , E.coli highest was at age of 51-60yrs(25.5%) followed by 41-50yrs(16.27%) , where as kilebsiella pneumonaiae highest ESBL isolates were in 71-80yrs(35.7%) followed by 61-70yrs(28.5%) as shown in fig 4 this showed the prevalence of kilebsiella pneumonaiae in the elderly age group when compared to other isolates .this was in contrast to that reported by (Akram M et al 2007).

Among all the antibiotics tested least resistance was shown by imipenem which was lower than that shown by (Dalela G et al 2012), , (Eshwarappa M et al 2014) but was higher than that shown by(Ritu agarwal et al 2009) .imipenem and meropenem belong to the Carbapenem group with almost the same resistance pattern they are extremely potent and broad spectrum βlactum antibiotic as it is resistant to most β- lactamases. When compared to imipenem , meropenem is good for urinary tract infections as it not hydrolysed by the renal peptidases shown in fig 5,6 & table 1.

Among the aminoglycosides tested amikacin showed the least resistance lower

Table.1 Comparative study of antibiotic sensitivity test

Antibiotics	study %	(Borthakur et al 2012) %	(Eshwarappa et al 2014) %	(Dalela et al 2012) %	(Ritu agarwal et al 2009) %
Imipenem	1.5	0	3.9	5.3	0
Amikacin	10.8	78.57	28	26.3	-
Gentamicin	63.1	-	49.2	-	-
Ciprofloxacin	86.2	69.04	74.1	-	-
Levofloxacin	81.5	-	-	-	-
Ofloxacin	83.1	-	74.1	-	25
Nitrofurantoin	32.3	61.9	28.6	42.1	68.65
Norfloxacin	81.5	-	74.1	-	74.62
Nalidixic acid	98.5	-	-	-	74.62

Figure.1

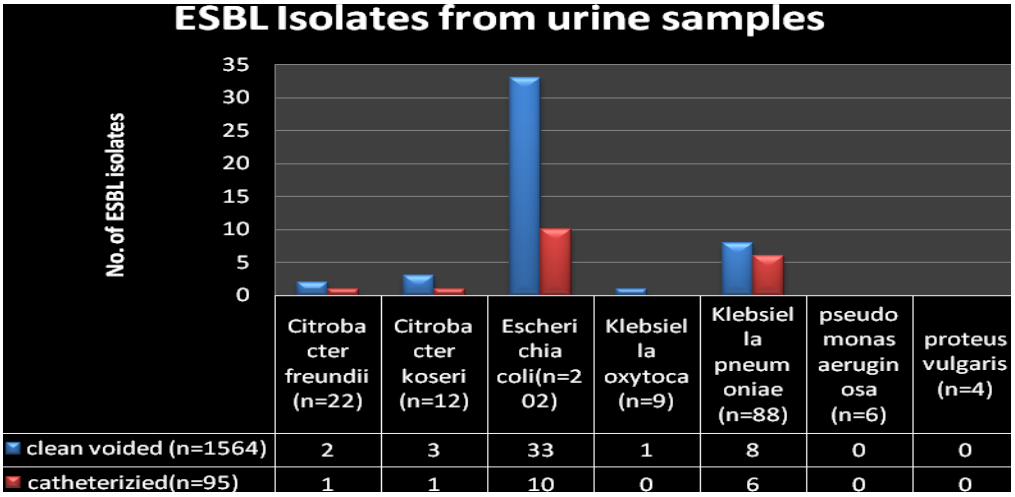


Figure 2

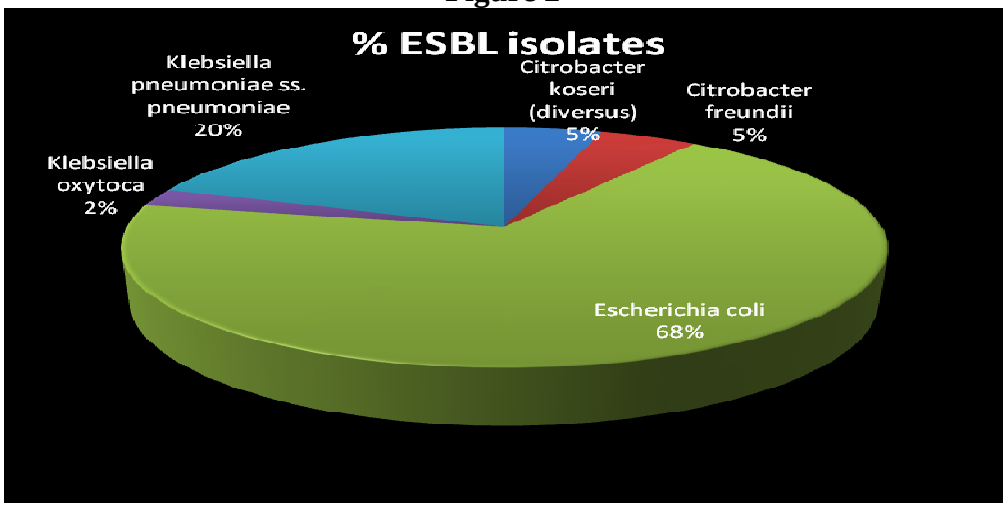


Figure.3

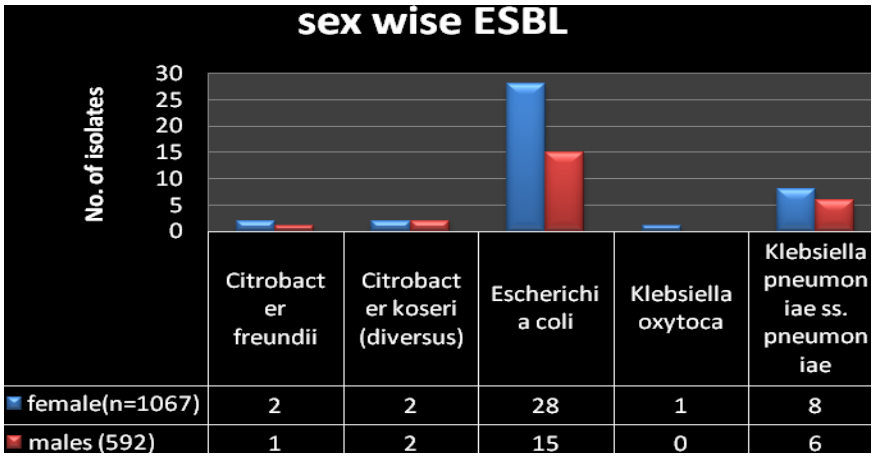


Figure.4

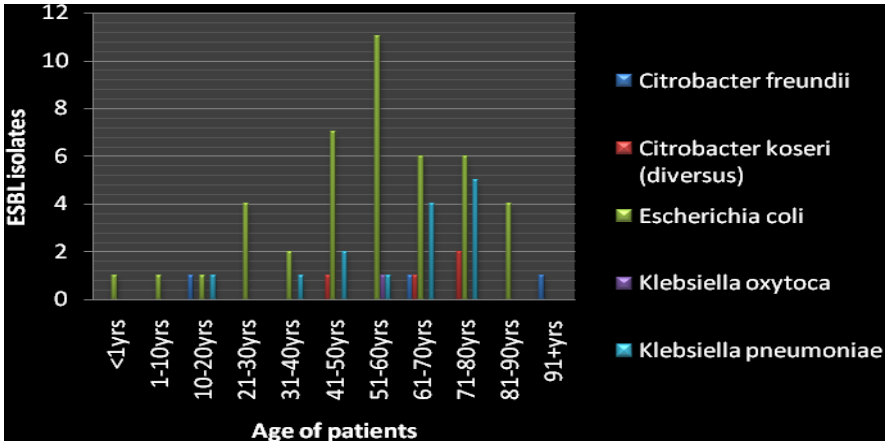


Figure.5

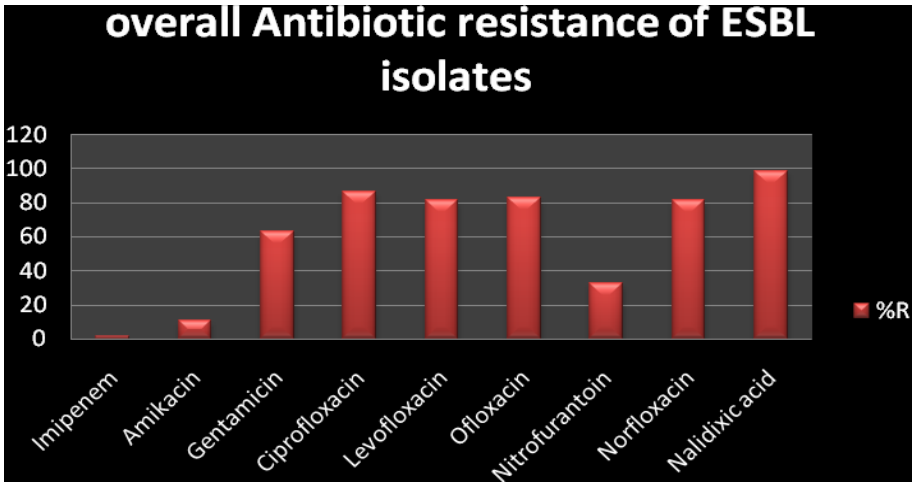
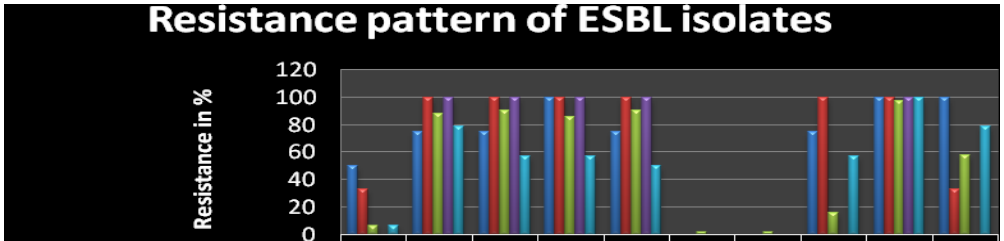


Figure. 6



	Amikacin	CIProfloxacin	Ofloxacin	Norfloxacin	Levofloxacin	Imipenem	Meropenem	Nitrofurantoin	Nalidixic acid	Gentamicin
Citrobacter koseri (diversus)	50	75	75	100	75	0	0	75	100	100
Citrobacter freundii	33.3	100	100	100	100	0	0	100	100	33.3
Escherichia coli	7	88.4	90.7	86	90.7	2.3	2.3	16.3	97.7	58.1
Klebsiella oxytoca	0	100	100	100	100	0	0	0	100	0
Klebsiella pneumoniae ss. pneumoniae	7.1	78.6	57.1	57.1	50	0	0	57.1	100	78.6

than that shown by (Dalela G et al 2012), (26.3%), (Eshwarappa M et al2014) (28%) and (A. K. Borthakur et al 2012) (78.57%) the sensitivity of amikacin when compared to other aminoglycosides is mainly its resistance to bacterial aminoglycosides inactivating enzymes it can be used as a reserved drug for hospital acquired gram negative bacillary infections where gentamicin and tobramycin resistance is high. Gentamicin resistance was higher than that shown by , (Eshwarappa M et al2014) as shown in fig 5,6 & table 1.

Among the urinary antiseptics tested nitrofurantoin resistnace was lower than that shown (Dalela G et al 2012), (A. K. Borthakur et al 2012) and (Ritu agarwal et al 2009) but higher than that shown by (Eshwarappa M et al2014) and was a better alternative to the other urinary antiseptics tested nalidixic acid whose resistance higher than that shown by(Ritu agarwal et al 2009).Bacterial resistance to nitrofurantion develops slowly when compared to nalidixic acid it can also antagonizes the action of nalidixic acid. nitrofurantoin is more active against infections caused by E.coli than other gram negative bacteria as shown in fig 5,6 & table 1 Among the flouroquinolones tested ciprofloxacin , ofloxacin , norfloxacin and levofloxacin the resistance shown were higher than that shown by (Eshwarappa M et al2014) ,(Ritu agarwal et al 2009) and (A. K. Borthakur et al 2012). Norfloxacin is a better alternativethan other flouroquinolones as it can attain better concentrations in the urine . Resistance is mainly due to chromosomal mutation producing a DNA gyrase or poisomerase iv with reduced affinity for flouroquinolones as shown in fig 5, 6 & table 1

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