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DETECTION OF ESBL-PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN PATIENT SUFFERING WITH SYMPTOMATIC URINARY TRACT INFECTION IN TERTIARY CARE HOSPITAL.

Microbiology

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ABSTRACT

Aim and objectives: This study is to determine the prevalence of Extended spectrum beta-lactamases (ESBLs) in urinary isolates. To compare different phenotypic methods for ESBL detection and to evaluate the antibiotic resistance patterns among ESBL producing urinary Escherichia coli.

Methodology: Urinary isolates of E.coli that were resistant to at least one of 3rd generation cephalosporins (cefotaxime, ceftazidime) were screened and tested for ESBL production using the Double disc diffusion test (DDDT), quantitative E-strip method and Automated Vitek-2.

Result: A total of 341 Klebsiella *pneumoniae* and Escherichia *coli* isolates were screened from patients with symptomatic UTI. 207 (60.7%) Escherichia *coli*, 134 (39.2%) Klebsiella *pneumonia*. ESBL production was detected in 48.8% and for 161 screening test positive E. coli, ESBL production was detected in 40%.

KEYWORDS

Extended spectrum beta-lactamases (ESBLs), ESBL E-strip test, Double disc diffusion test (DDDT).

Introduction:

Urinary tract infections (UTIs) remain the most common bacterial infection in human. Among the wide array of antibiotics beta-lactamase are the most varied and most widely used agents, accounting over for 50% of all systemic antibiotics in use (Shahi SK, 2013**).** β-Lactam antibiotics are commonly used to treat bacterial infections, The antibiotics includes penicillin, cephalosporin, monobactam and carbapenem groups. The uses of these antibiotics are rising mainly the third generation of cephalosporins, have been responsible for the emergence of beta lactamases mediated resistance, which leads to the development of ESBL producing bacterial resistance. ESBLs, are the enzymes that mediates resistance to extended spectrum drugs e.g., third generation cephalosporins as well as monobactams such as aztreonam, (CLSI, 2014). These enzymes are responsible to catalyze hydrolysis of the Beta-lactam ring of antibiotic, therefore destroys the antimicrobial activity. The emergence of ESBLs have been reported in many different genera of enterobactericeae and non fermenters worldwide (Friedman C, 2005) However, these are most commonly isolated in Klebsiella *pneumoniae* & Escherichia *coli*. **(**Shashi SK, 2013**)** Organisms producing ESBL are also resistant to many of other classes of antibiotics, as the resistance is plasmid mediated with the gene encoding ESBLs that often carry resistance determinants. Initially, ESBL producing organisms were isolated from hospital acquired infections but the organisms of particular resistance are now also being isolated from community also (Shakil S 2016). The colonization rate for K. pneumonia is low in healthy individuals in general population. But it is observed that the rate of ESBL producing Klebsiella *pneumoniae* is much high in patients admitted to hospitals taking long term health. The rapid spread of resistance to broad-spectrum beta-lactam antibiotics in pathogenic strains of bacteria has recently been major health care problem worldwide. The emergence of extended-spectrum Beta-lactamase (ESBL) producing bacteria, specifically in Escherichia *coli* and Klebsiella *pneumoniae*, has now been a critical problem for the development of treatment against bacterial infection.

MATERIAL AND METHODS

The prospective study was conducted in the Department of Microbiology, Maharani Laxmi Bai Medical College, Jhansi (U.P), India. A total of, 341 Klebsiella *pneumoniae* and Escherichia *coli* isolates were identified from patients with symptomatic UTI. Mid-stream urine sample was collected in a sterile disposable container (Uricol, Hi-Media) and processed within 1 hr. A semi-quantitative loop (Hi- Media Laboratories) measuring 2.2 mm in diameter with holding capacity of 0.005 ML was employed to culture urine on CLED agar, blood agar and MacConkey agar plates. The culture plate was incubated at 37°C for overnight (Singh S, 2017). Antibiotics Susceptibility was tested by Kirby-Bauer disc diffusion method and Vitek-2 (BIOMERIEUX) automated system (Card number 280) using these antibiotics, Ampicillin, Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Cefuroxime, Ceftriaxone/sulbactam, Ceftazidime, Cefepime, Aztreonam, Doripenem, Imipenem, meropenem, Amikacin, Gentamicin, Micocycline,Tigecycline, Colistin, Trimethoprim/Sulfamethoxazole.

Disc Susceptibility test for screening of ESBLs

All isolates was Screened for ESBL detection using two indicator Cephalosporins namely Ceftazidime (30 µg) , Cefotaxime (30 µg). The isolates was considerd to resistant if the inhibition zone size for ceftazidime, cefotaxime was ≤22 mm and ≤27. The strains shows that resistant to at least one of the two cephalosporins was tested and further confirmation by other phenotypic and automated method.

Phenotypic confirmatory method for detection of ESBLs

1.COMBINATION DISC DIFFUSION METHOD

This test requires the use of a third-generation cephalosporin antibiotic disc alone and in combination with clavulanic acid. In this study, two combinations were used, disc of Ceftazidime (30µg) alone and a disc of Ceftazidime + Clavulanic acid (CAC) and Cefotaxime(30µg) alone and a disc of Cefotaxime+Clavulanic acid (30µg/10µg). Both the discs were placed at least 25 mm apart center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid were measure. ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.

2.ESBL E-test:

The E- test ESBL Strip contains a double sided seven-dilution range of Cefotaxime(CTX) (0.25- 16 µg/ml) and Cefotaxime + clavulanic acid (CTX+) (0.016-1 µg/ml) in combination with a fixed concentration for ESBL production. The E-test was done according to manufacturer's instructions. (Himedia EM099) positive and negative controls were used. MIC ratio of CTX(Cefotaxime)/ CTX+(Cefotaxime + Clavulanic acid) were interpreted.

3.VITEK- 2 SYSTEM

The Vitek 2 System (bioMérieux) is a rapid semi-automated microbiological system used for bacteria identification, minimum inhibitory concentration determination, antimicrobial susceptibility testing (AST), and resistance mechanism. Detection of ESBL by VITEK- 2 is a phenotypic confirmatory tool for rapid detection of ESBL-producing organism.

Results and Discussion

A total of 1169 urine samples were collected from various clinical Departments including OPD and IPD. Out of them, 207 Escherichia

coli, 134 Klebsiella *pneumoniae* and 398 other clinical pathogens (including Klebsiella oxytoca, Staphylococcus aureus, CONS, proteus species, Citrobacter speices, Pseudomonas species, Acinetobacter species , Candida species) were isolated. ESBL screening test was performed. Of the 90 screening test positive K. pneumoniae isolates, ESBL production was detected in 48.8% and for 161 screening test positive E. coli, ESBL production was detected in 40%.[Table-1]

Table-1 ESBL screening

Organisms	Positive	Negative
Escherichia *coli* (207)	161 (77.77%)	46(22.22%)
Klebsiella *pneumoniae* (134)	90 (67%)	44(32.83%)

Out of 161 *E. coli* isolates that were positive in the screening test, 59 (36.6%) were phenotypically confirmed by double disc diffusion method,62 (38.5**%)** were confirmed by E- test and 65(40%) were confirmed by vitek-2 system as ESBL producers. Out of the 90 K. *pneumoniae* isolates, 42 (46.6%) were identified by double disc diffusion method,44 (48.8%) were confirmed by E- test and 44 (48.8%) were confirmed by vitek-2 system as ESBL producers.[Table-2]

Table-3 indicates that imipenem and amikacin is the drug of choice for the management of ESBL-producing organisms. All the isolates were resistant to third generation cepahalosporins. Nitrofurantoin and fosfomycin can also be given for treatment of UTI.

Table-2: Detection of ESBL by different phenotypic methods.

Organism	Double disc diffusion test(DDST)	E- test	Vitek-2 system
Escherichia *coli*	59 (36.6%)	62 (38.5**%)**	65 (40%)
Klebsiella *pneumoniae*	42 (46.6%)	44 (48.8%)	44 (48.8%)

Table-3: Antimicrobial Susceptibility among all Screened Positive Isolates (n=251).

Antimicrobial Agent	No. (%) of Susceptible Isolates
Ampicillin	0(0.0)
Amoxicillin/Clavulanic acid	29 (11.5)
Piperacillin	0(0.0)
Piperacillin/Tazobactam	79(31.47)
Cefazolin	0(0.0)
Cefoxitin	185(73.8)
Cefotaxime	0(0.0)
Ceftazidime	0(0.0)
Cefepime	0(0.0)
Imipenem	248 (98.8)
Amikacin	236 (94.1)
Gentamicin	51 (20.3)
Ciprofloxacin	48 (19.1)
Norfloxacin	79(31.7)
Tetracycline	48(19.8)
Nitrofurantoin	96(38.2)
Trimethoprim/ Sulphamethoxazole	1 5(5.9)

Conclusion

It should be done routine microscopy for urine because absence of pus cell with growth of organism on culture there is no significance of culture. ESBL producing *E. coli and K. pneumoniae* is a serious concern for the hospitals. It is associated with high morbidity among paediatric patients which results in high cost of treatment. An appropriate antimicrobial therapy can only be started timely with the early detection of ESBLs. The CLSI confirmatory test can be used reliably especially when the facilities for molecular charaterization are not available. The detection of ESBL producing strains will also help to establish and implement a strict infection control policy to stop the spread of ESBL *E. coli and K. pneumoniae*.

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