

Original Research Article

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A Study by Double Disc Diffusion (DDDT) Method to Compare Ceftazidime + Clavulanic Acid and Cefotaxime + Clavulanic Acid for Detection of Extended Spectrum B- Lactamase among *Escherichia coli* and *Klebsiella pneumoniae* in Urinary Isolates

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ABSTRACT

The detection of extended- spectrum β -lactamase (ESBL) producing bacteria is important for infection control and epidemiological surveillance. The purpose of the present study was to compare two phenotypic methods for detection of ESBL positive *Escherichia coli* and *Klebsiella pneumoniae* in urinary isolates. This study was carried out in the Department of Microbiology, Rama Medical College, Kanpur(U.P), India from November 2015 to August 2016. Patients of all ages and genders were taken as study population. Lactose fermenting gram negative bacilli(GNB) were analyzed and routine susceptibility testing was performed. Screening test for ESBL production was done by two disc diffusion methods Ceftazidime and Ceftazidime+Clavulinic acid(CAZ/CAZC) and Cefotaxime and Cefotaxime+ Clavulinic acid (CTX/CTXC). In our study, 576 urine samples have been collected, from them 369 uropathogens were isolated 102 (17.07%) *Escherichia coli*, 66 (11.45%) *Klebsiella pneumoniae* and 201 others(34.89%) (including *Staphylococcus aureus*, CONS, *Proteus* species, *Pseudomonas aeruginosa*, *Acinetobacter* species and candida species) respectively. The prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* in our study is 30.39% and 36.36% respectively. Of the 65 *Escherichia coli* and 41 *Klebsiella pneumoniae* Screened For ESBL . 46 isolate were detected by combined disc method. The use of ceftazidime(30 μ g) and ceftazidime + clavulanic acid(30 μ g/ 10 μ g) detected 46 isolates as ESBL positive as compared to cefotaxime(30 μ g) and cefotaxime + clavulanic acid(30 μ g/ 10 μ g) which detected 33 isolates as ESBL positive. The use of ceftazidime(30 μ g) and ceftazidime + clavulanic acid(30 μ g/ 10 μ g) DDDT phenotypic methods are useful diagnostic tool for detection of ESBL in gram negative bacilli..

Keywords

Extended-spectrum β -lactamase, Phenotypic detection, double disc diffusion test.

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Introduction

Extended spectrum β -Lactamases (ESBL) are enzymes produced by pathogens belonging to Enterobacteriaceae, most commonly *Escherichia coli* and *Klebsiella pneumoniae* (Kenneth *et al.*, 2010).

They cause serious infections and have high mortality rates. Multidrug-resistant ESBL *E. coli* (CTX-M enzyme type) has emerged as an important cause of UTI (Gisele *et al.*, 2010). ESBL strains are capable of efficiently hydrolyzing many beta-lactam antibiotics

including 3rd generation cephalosporins and monobactams. β -lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) generally inhibit ESBL producing strains (Rupp, 2003). It is increasingly being reported that they are acquiring a transmissible form of antibiotic resistance. This indicate that penicillins and cephalosporins which have been used for many years are no more effective against ESBL positive (Duttaroy *et al.*, 2005). Nosocomial outbreaks of infections caused by ESBL-producing gram-negative bacteria have also been reported, which are mainly the result of extensive and inappropriate use of third-generation cephalosporins. The major risk factors implicated are long-term exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, instrumentation, or catheterization (Kumar *et al.*, 2006).

The aim of this study is to evaluate the utility of ceftazidime+clavulanic acid as a better method than cefotaxime(30 μ g) and cefotaxime + clavulanic acid(30 μ g/ 10 μ g) for detection of extended spectrum beta lactamases (ESBL) in *E.coli* and *Klebsiella pneumoniae* in urinary isolates.

Materials and Methods

The present study was conducted in the Department of Microbiology, Rama Medical College, Kanpur over a period of 10 months (November 2015 to August 2016). A total of 576 urine samples were obtained from various clinical departments including OPD and IPD of all age groups including both the genders. All samples were routinely cultured on CLED agar plates at 37°C aerobically for 18 hours and gram negative isolates were further characterized by standard biochemical tests. Isolates were screened for ESBL production by using disc diffusion test on Muller Hinton agar according to CLSI guidelines, 2016.^[16]

Isolates showing inhibition zone size of ≥ 22 mm with ceftazidime (30 μ g), ≥ 25 mm with ceftriaxone (30 μ g), ≥ 27 mm with cefotaxime (30 μ g), ≥ 27 mm with aztreonam (30 μ g) were suspected for ESBL production. All screening test positive isolates were further confirmed by Phenotypic confirmatory test for ESBL producers by double disc diffusion test (DDDT) and Double disc diffusion test (DDDT) was performed by two methods which were ceftazidime and ceftazidime plus clavulanic acid (CAZ/CAZC) and cefotaxime and cefotaxime plus clavulanic Acid (CTX/CTXC). *E. coli* ATCC 25922 was used as negative control and *Klebsiella pneumoniae* ATCC 700603 used as positive control.

Results and Discussion

In our study, 576 urine samples have been collected, from them 369 uropathogens were isolated 102 *Escherichia coli*, 66 *Klebsiella pneumoniae* and 201 other isolates (including *Staphylococcus aureus*, CONS, Proteus species, *Pseudomonas aeruginosa*, *Acinetobacter* species and candida) respectively.[Table-1] The prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* in our study, is 30.39% and 36.36% respectively. The proportion of ESBL was found higher in *Klebsiella pneumoniae*. [Table-2] The ESBL frequencies varied from 66.7% in India, 54.7% - 61% in Turkey (Gaurav) 41% in United Arab Emirates, 31.7% in Kuwait and 72.1% in Iran but was found lower in America and Europe (Kumar *et al.*, 2006).

The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different parts in India. In our study, the rate of ESBLs-producer isolates was higher than that reported from Nagpur, Aligarh respectively in 2004, 2009 and 2012 (Lal *et al.*, 2007;

Shoorashetty *et al.*, 2011; Tankhiwale *et al.*, 2004). Whereas still lower than the rate reported in a study from Delhi, Jaipur and

Maharashtra respectively in 2003, 2014 and 2016 in urinary isolates.

Table.1 Distribution of organisms from the urine samples

Total urine sample	<i>E.coli</i>	<i>Klebsiella pneumoniae</i>	Others	Total Urinary pathogens (%)
576	102(17.07%)	66(11.45%)	201(34.89%)	369(64.06%)

Table.2 ESBL screening method

Organisms	Positive	Negative
<i>Escherichia coli</i>	65	37
<i>Klebsiella pneumoniae</i>	41	25

Table.3 Comparison between different disk diffusion methods to detect ESBL

Total isolates	Organisms	Screening test positive	Ceftazidime and Ceftazidime with clavulanic acid	Cefotaxime and Cefotaxime with clavulanic acid
102	<i>Escherichia coli</i>	65	27	18
66	<i>Klebsiella pneumoniae</i>	41	19	15

Fig.1 showing ESBL production by phenotypic confirmatory method (by Double Disk Diffusion test)



This may be due to differences in the type and volume of consumption of antibiotics and differences in the year of collection of isolates. However, it reflects the increase in the prevalence of ESBL producers in India.

As in other studies, the majority of ESBL-producing isolates were recovered from urine. Of the 65 *Escherichia coli* and 41 *Klebsiella pneumoniae* isolated by ESBL screening test among them, a total of 46 ESBL were

detected by combined disc method. [Table-3] The use of ceftazidime (30 µg) and ceftazidime + clavulanic acid(30µg/ 10µg) detected 46 isolates as ESBL positive as compared to cefotaxime(30 µg) and cefotaxime + clavulanic acid(30µg/ 10µg) [Table-3] [figure-1] which detected 33 isolates as ESBL positive. The result of our study shows similar findings with those who reported 45.2% isolates were ESBL positive by using ceftazidime and ceftazidime + clavulanic acid as compared to cefotaxime and cefotaxime + clavulanic acid which detected 36% isolates as ESBL positive.

In conclusion routine use of both DDDT is useful for the detection of ESBL in clinical samples. The use of Ceftazidime and Ceftazidime with clavulanic acid was found to be the better method for detection of ESBL producing bacteria in our study especially in urine isolates (*E.coli* and *Klebsiella pneumoniae*).

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