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Original Research Article

Incidence of Urinary ESBL'S at a Teritiary Care Hospital

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Abstract: Gram-negative bacilli (GNB) are commonly implicated in clinical diseases. However, with their increasing resistance to antimicrobial agents, treatment becomes a challenge. The increasing frequency and antibiotic resistance among extended-spectrum β-lactamases (ESBLs)-producing bacteria are posing a serious threat. ESBL producers most often acquire and exhibit additional resistance to other antimicrobials such as Quinolones, Cotrimoxazole, Trimethoprim, Tetracycline's and Aminoglycosides, thus making the therapeutic options very limited. The present study was conducted in a tertiary care hospital from Jan2016-June2016. ESBL screening was done as per Clinical Laboratory Standards Institute (CLSI) guidelines. A total of 889 urinary isolates were included in the study, of which 368 were Klebsiella pneumoniae and 195 Were Escherichia coli, these were subjected for ESBL screening by Kirby-Bauer method and confirmed by Disk potentiation method & Double Disc synergy method and 34% of Klebsiella pneumoniae & 45% of E.coli were found to be ESBL producers. Imipenem susceptibility was 100%. Over 90% of ESBL isolates showed resistance to Aztreonam and Cephalosporins .Resistance to Aminoglycosides and Quinolones was relatively low. Imipenem should be reserved for life threatening infections. Periodic susceptibility studies will help the physicians in choosing empirical therapy and preserve the higher antimicrobials to life threatening infections.
Keywords: Escherichia coli; Klebsiella pneumoniae; extended-spectrum beta-lactamases; susceptibility; antibiotics, Clinical Laboratory Standards Institute

INTRODUCTION:

The precise definition of the term Extended-Spectrum β -Lactamase (ESBL) remains unclear but is generally used to refer to any β -lactamase, generally acquired rather than inherent to a species, that is either able to confer resistance to oxyimino- cephalosporins (but not carbapenems), or that has an increased ability to do so, as compared with classic members of its genetic family. ESBL producing strains of Enterobacteriaceae particular among Klebsiella pneumoniae and Escherichia coli (E. coli) have emerged as a major problem in hospitalized as well as community based patients [1]. Infections associated with ESBL producing isolates are difficult to detect and treat, thereby causing increased mortality and morbidity of patients [2] β -lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins [3]. E. coli possess a naturally occurring chromosomally mediated plasmid mediated -lactamases. These enzymes are thought to have evolved from penicillin binding protein. Detection of ESBL producers may be of utmost importance because this represents an epidemiologic marker of colonization and therefore there is potential for transfer of such organisms to other patients [4]. Knowledge of local prevalence and antibiotic susceptibility pattern will help the physician in choosing empirical therapy and help in formulating hospital antibiotic policy. Hence the present study was done to know the prevalence and antibiogram of ESBL producing E. Coli & Klebsiella pneumonia from Urinary samples.

MATERIAL AND METHODS:

Urine samples collected in universal container from patients either admitted to different wards or attending the outpatient clinics were included. Samples were inoculated using an inoculating loop of 10 µL volume on the blood agar plates and MacConkey agar plates. Identification of the isolated organism was done on the basis of routine biochemical tests and Gram staining was performed to confirm E. Coli & Klebsiella using standard protocol. .All inoculation processes of the samples were performed under aseptic techniques.

Consecutive non-duplicate K. pneumoniae and E. coli isolates were screened for ESBLs as per CLSI

guidelines[5] . Isolates showing inhibition zone size of <22 mm with Ceftazidime (30 µg), and <27 mm with Cefotaxime (30 µg) were processed for confirmatory tests.

Confirmatory tests for ESBLs

a) **Double Disc Synergy Method**: 30 µg antibiotic disks of Ceftazidime and Cefotaxime are placed on the lawn culture plate, 30 mm (center to center) from the Amoxicillin/clavulanic acid (20/10 µg) disk. This plate was incubated aerobically overnight at 37°C and examined for an extension of the edge of zone of inhibition of antibiotic disks toward the disk containing Clavulanate. It is interpreted as synergy, indicating the presence of an ESBL.(FIG-1)

b) **Disk Potentiation Method**: with combination disk of Ceftazidime plus clavulanic acid (30/10 mcg) and Cefotaxime plus clavulanic acid (30/10 mcg) discs were also included along with Ceftazidime (30 mcg) and cefotaxime (30 mcg) discs on Muller-Hinton agar. Organism was considered as ESBL producer if there was an increase of >5 mm in the zone diameter of Ceftazidime/clavulanic acid disc with respect to that of Ceftazidime disc alone and or ≤5 mm increase in the zone diameter of Cefotaxime/clavulanic acid disc with respect to that of Cefotaxime disc alone.(FIG-2)

Fig-1: Double Disk Synergy Method

Fig-2: Disk Potentiation Method

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines .Commercially available antibiotic disks (Hi Media India) were used for antimicrobial susceptibility testing. The following antibiotic disks were used, Ampicillin (10 µg), Amoxicillin/clavulanic acid (20/10µg) Ceftazidime (30 µg), Cefotaxime (30 µg), Aztreonam (30 µg), Imipenem (10 µg), Amikacin (30 µg) and Ciprofloxacin (30 µg), E.coli ATCC 25922 and E. coli ATCC 35218(for β lactam/ β lactamase inhibitor combination) were used as control strains.

RESULTS & DISCUSSION:

With the exception of one E. coli isolate, there was 100% agreement between the two confirmatory tests for ESBL detection .There was a slight female preponderance in the outpatient group, and the majority of the hospitalized patients were older than 18 years of age. During the study period, a total of 889 Gram negative bacteria were isolated, including 193(21%) strains of E. coli and 368(41%) strains of K. pneumoniae. Among these, there were 215 (38%) ESBL isolates during the study period, of which 88 (45%) were E. coli and 127 (34%) were K. pneumonia(Table-1). The antibiotic resistance pattern exhibited by ESBL producers and NON-ESBL producers were given in (Table-2).

Table-1: Distribution Of Isolates & Esbl's

TOTAL GRAM NEGATIVE ISOLATES	TOTAL E.coli isolated	TOTAL K.peumoniea isolated	ESBL E.coli	ESBL K.peumoniea
889	193(21%)	368(41%)	88(45%)	127(34%)

Table-2: Antibiogram of E. Coli & K. pneumoniae. (Resistance pattern)

Antibiotic	ESBL producers (n=215)	NON ESBL producers (n=346)
Amikacin	4(1.8%)	2(0.5%)
Amoxyclav	7(3.2%)	4(1.2%)
Aztreonam	17(7.9%)	0
Ciprofloxacin	7(3.2%)	2(0.5%)
Ceftazidime	14(6.5%)	6(1.7%)
Cefotaxime	11(5.1%)	4(1.1%)
Imipenem	0	0

The presence of ESBL enables certain Gram-negative bacteria to inactivate extended-spectrum (third-generation) cephalosporins, broad-spectrum penicillins and monobactams but do not affect the cephamycins or carbapenems. As the presence of these enzymes significantly impacts the efficacy of β -lactam therapy, there is a need for clinical laboratories to accurately recognize ESBL producers so as to better support therapy and provide accurate prevalence data. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years, resulting in limitations of therapeutic options [6] .Highest resistance among ESBL producers was seen with ampicillin and least resistance with Imipenem. Resistance to Cephalosporin group ranged from 77% to 83%. Resistance to aminoglycosides and nitrofurantoin was relatively low. This finding is similar to other studies [7, 8]

CONCLUSION

This study shows the prevalence of ESBL in urine samples among E. coli and K. pneumoniae isolates. Amikacin, Quinolones and carbapenems remain the most useful drugs for treatment of ESBL infections. However occurrence of multidrug resistance strains is of major concern. Large surveys, continued surveillance by clinical microbiology laboratories, judicious use of antimicrobial agents as well as implementation of infection control measures are recommended if the frequency of ESBL isolates is to be reduced in hospital setting.

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