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Full Length Research Article

Evaluation of Extended Spectrum Beta Lactamase in Gram Negative Urinary Isolates

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ABSTRACT

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Extended spectrum beta lactamases hydrolyze expanded spectrum Cephalosporins like Ceftazidime, Cefotaxime which are used in the treatment of urinary tract infections (UTI).A prospective study was undertaken to know the occurrence of ESBL and their antibiotic susceptibility pattern to guide empirical therapy for UTI. Over a period of three months (March 15 to May 15), organisms grown in pure culture and in significant numbers from urine sample were identified by standard biochemical tests and antibiotic susceptibility determined by disc diffusion method. Gram-negative bacilli that were resistant to third generation Cephalosporins were further tested for ESBL production by Combination disk test method. MIC of Ceftazidime, Cefotaxime and Cefepime was determined for ESBL producing Gram negative isolates by an Epsilometer test (E test). Of the 272 isolates, 185(68%) were resistant to third generation cepholosporines. Of these, 116(43%) were found to be ESBL producers. Based on the species79 (50%) *Esch. coli*, 13 (32%) *Klebsiella pneumoniae, 9 (24%) Pseudomonas aeruginosa, 6(43%) Citrobacter freundii, 4(50% Acinetobacter sp, 4(50%) Proteus sp and 1(20%) Enterobacter aerogens* harboured ESBL enzymes. Most of the ESBL producing isolates were multidrug resistant. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

Key words: Extended spectrum β-lactamases, Urinary tract infection.

INTRODUCTION

Urinary tract infection represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group (Jharna mandal, 2012). In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections (Manikandan *et al.,* 2011). The most common mechanism of resistance in Gram negative bacteria is by the production of ß lactamases which inactivate ß lactam antibiotics. Among the ß lactamases, Extended Spectrum ß lactamases (ESBL) and Amp C ß-lactamases are most commonly produced. Extended-spectrum β-lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation Cephalosporins and Aztreonam but are inhibited by Clavulanic acid (Varsha Gupta. 2007). The total number of ESBLs now characterized exceeds 200. ESBLs are often encoded by genes located on large plasmids, and these also carry genes for resistance to other antimicrobial agents such as Aminoglycosides, trimethoprim, sulphonamides, Tetracyclines and Chloramphenicol. Recent studies have demonstrated Fluoroquinolone resistance mediated by co-transfer of the qnr determinant on ESBL-producing plasmids. Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing enterobacterial isolates (Deepti Rawat and Deepthi Nair, 2010). As a result, ESBL-producing organisms pose a major problem for clinical therapeutics. Thus there is need for efficient infection-control practices for containment of outbreaks; and intervention strategies. Hence the present Study aimed at gaining knowledge about the susceptibility patterns of Gram negative pathogens responsible for UTIs and their extended spectrum β lactamase resistance may help the clinicians to choose the right empirical treatment.

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MATERIALS AND METHODS

A total of 272 concecutive Gram negative urinary isolates of species *Esch. coli* (n=158), *Klebsiella pneumonia* (n=41), *pseudomonas aeruginosa (n=38), Citrobacter sp* (n=14), *Acinetobacter sp (n=8), Proteus sp (*n=8) *and Enterobacter aerogens (n=5*) obtained over a period 3 months from March 2015 to May 2015 at Govt.Thoothukudi medical collge hospital (Tamilnadu) identified by standard method were included in the present study. Susceptibility to antibiotics (concentration in µg) Ampicillin (10), Gentamycin (10), Amikacin (30), Cefotaxime (30), Ceftazidime (30), Ceftriaxone (30), Ceftazidime (30) and (Ciprofloxacin) (5) (Hi Media) were tested by Kirby Bauer's disc diffusion method and interpreted as Clincal Laboratory Standard (CLSI) recommendations.

Detection of ESBL production

Gram negative isolates resistant to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime, & Ceftriaxone) were tested for ESBL production by combination disk test method as per CLSI guidelines. Phenotypic confirmatory test. (Combination disk test method) (Paul, 2010). Ceftazidime (30µg) disk and a Ceftazidime plus Clavulanic acid (Ca 30 µg + Caz 10 µg) disk were placed at a distance of 20 mm apart on a lawn of culture of the suspected ESBL producing clinical isolates on Muller Hinton Agar. The plates were incubated at 37°C overnight.

Interpretation

The test organism was considered to produce ESBL if the zone size around the ceftazidime plus clavulanic acid increased >5 mm in comparison to the third generation ceftazidime disk alone. This increase occurred because the β lactamases produced by the isolates were inactivated by clavulanic acid.

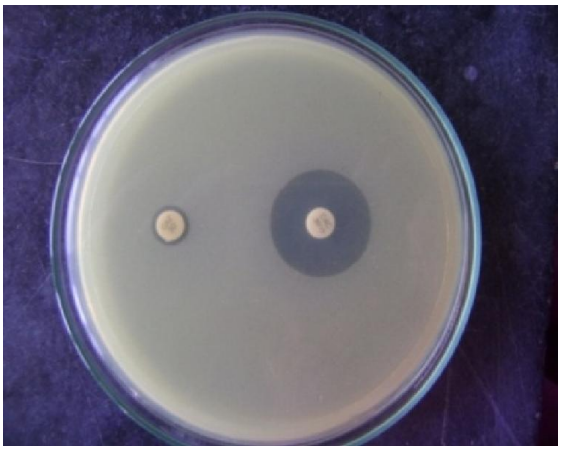

Figure 1. Combination disk test method for ESBL production

Minimum Inhibitory Concentration determination

MIC of Cefotaxime & Ceftazidime was determined for ESBL producing isolates by an Epsilometer test (E test). A saline suspension of each isolate was adjusted to a McFarland standard of 0.5 and inoculated over the surface of the Muller Hinton agar plate.

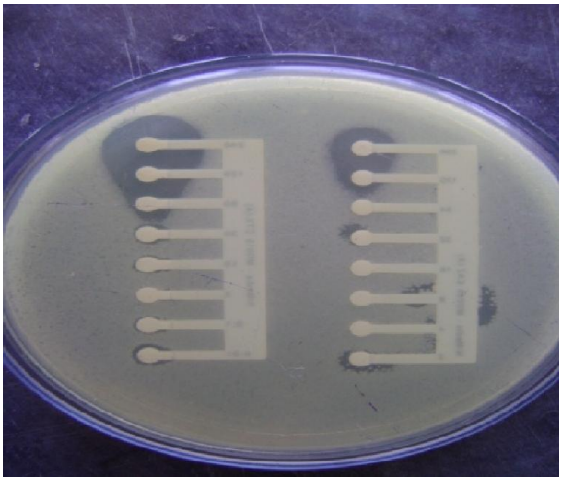

Figure 2. MIC determination by E test

The Himedia Hicomb MIC strip was applied on the agar surface using sterile forcep. Then the plates were incubated for 18 hrs at 35°C and the MIC was the point where the elliptical zone of growth inhibition intersected the MIC scale on the E test strip. The concentration range of antimicrobial on the E test strip corresponds to two fold dilutions in a conventional MIC method.

RESULTS

The antibiotic susceptibility testing for Gram negative urinary bacilli revealed 100% sensitivity to Imepenam followed by 88% to Amikacin,79% to Gentamycin and 55% to Ciprofloxacin. Of the 272 isolates tested, 185(68%) were resistant to third generation cephalosporins (Cefotaxime, Ceftazidime, Ceftriaxone). These isolates were tested for ESBL production by Combination disk test method. Out of 185 third generation Cephalosporins resistant isolates, 116 were positive for Extended spectrum β lactamase production. Out of 116 (43%) ESBL positive Gram negative bacteria 79 were (50%) *Esch.coli*, 13 (32%) were *Klebsiella pneumoniae, 9 (24%) were pseudomonas aeruginosa, 6(43%) were Citrobacter freundii, 4 (50%) were Proteus sp and 1 (20%) was Enterobacter aerogens.* ESBL producing Gram negative isolates were further subjected to MIC determination for Ceftazidime and Cefotaxime by Epsilometer test strip. MIC of Ceftazidime and Cefotaxime was ≥32 µg /ml for ESBL producing Gram negative isolates as per revised CLSI standards.

Table 1: Distribution of ESBL producers

Name of the organism	Total no of organism	No &% of 3GC resistance	No and % of ESBL producers
Escherichia coli	158	111(70%)	79(50%)
Klebsiella pneumoniae	41	28(68%)	13(32%)
Pseudomonas aeruginosa	38	20(53%)	9(54%)
Citrobacter sp	14	12(86%)	6(43%)
Acinetobacter sp	8	5(63%)	4(50%)
Proteus sp	8	6(75%)	4(50%)
Enterobacter sp	5	3(60%)	1(20%)
Total	272	185(68%)	116(43%)

DISCUSSION

This study demonstrates the presence of ESBL-mediated resistance in gram-negative urinary bacilli .ESBL detection is not commonly carried out in many microbiology units in developing countries. This could be attributed to lack of awareness and lack of resources and facilities to conduct ESBL identification (Dechen C Tsering *et al.,* 2009). The high rate (68%) of third generation Cephalosporin resistance noted among the Gram negative urinary isolates in the present study, similar findings were reported by Bobak Pourakbari *et al.* 2012. The prevalence of ESBL producers varies across continents and countries and also within hospitals. In India, the prevalence rate varies in different institutions from 28 to 84%. In the present study, ESBL production was observed in 43% of isolates. These findings were lower than the results published by NK.Debata et al (51.78%) (Debata, 2013) but higher than the result published by Kargar *et al.* ((11.75 %) (Mehdi Kargar *et al.,* 2014). In this study, ESBL producing isolates were significantly more resistant to Ciprofloxacin and Gentamicin as compared to non-ESBL producing gram-negative isolates. In our study, resistance to 3GCs was found to coexist with resistance to two or more antibiotics like Ciprofloxacin, Gentamicin as also reported by Subha *et al.,*

2003 and indicating multidrug resistance pattern. Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids. The spread of ESBL-producing bacteria has been expanding rapidly worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Therapeutic options against infections due to ESBL producers have also become increasingly limited. Health care interactions including the use of antibiotics, particularly oxyiminocephalosporins, and hospital transfers are among the well-defined risk factors for the acquisition of ESBL-producing bacteria.

Conclusion

The prevalence of ESBL was found to be 43% in our hospital which cannot be ignored. ESBLS constitute a serious threat to currently available antibiotics. Institutional outbreaks are increasing because of selective pressure due to heavy use of expanded spectrum Cephalosporins and lapses in effective control measures. So vigilance and timely recognition of infection with resistant bacteria and appropriate antibiotic therapy, is the only answer to the current multi drug resistant bacterial population.

REFERENCES

BobakPourakbari, Faad Ferdosian, Shima Mahmoudi 2012. Increase resistance rates and ESBL production between E.coli isolates causing urinary tract infection in young patient from Iran. *Brazilian Journal of Microbiology*, 766-769.

Debata, N. K., J. Jena, E Subudhi 2013. Prevalence of extended-spectrum-beta-lactamase and metallo-beta-lactamase producing multi drug resistance gram- negative bacteria from urinary isolates. *Indian Journal of Medical Microbiology*, Vol. 31, No. 4, pp. 420-421

Dechen C. Tsering, Shyamasree Das, Luna Adhiakari, Ranabir Pal, and Takhellambam SK. Singh, 2009. Extended Spectrum Beta-lactamase Detection in Gram-negative Bacilli of Nosocomial *Origin. J Glob Infect Dis*., Jul-Dec; 1(2): 87–92

Deepti Rawat and Deepthi Nair, 2010. Extended-spectrum β-lactamases in Gram Negative Bacteria. *J Glob Infect Dis*.; 2(3): 263–274.

Jharna Mandal, N. Srinivas Acharya, 2012. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli. Indian J Med Research*, 136, P 842-849.

Manikandan, S., S. Ganesapandian, Manoj Singh and A. K. Kumaraguru, 2011. Emerging of Multidrug Resistance Human Pathogens from Urinary Tract Infections. *Current Research in Bacteriology*, 4: 9-15.

Mehdi Kargar, Mohammad Kargar, Mohammad Zareian Jahromi, Akram Najafi, Sadegh Ghorbani-Dalini, 2014. Molecular detection of ESBLs production and antibiotic resistance patterns in Gram negative bacilli isolated from urinary tract infections. *Indian J Pathol Microbiol,* 57: 244-8

Paul, C. Schreckenberger, 2010. Changes in the CLSI standards for antibiotic susceptibility testing for 2010.

Subha, A., V. Renuka Devi and S. Ananthan, Amp, C. 2003. β lactamase producing multidrug resistant strains of Klebsiella spp. & Escherichia coli isolated from children under five in Chennai, *Indian J Med Res*, 117, pp 13-18.

Varsha Gupta, 2007. An update on newer β lactamases. *Indian J Med Res*, 126, pp 417-427

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