VOLUME-6 | ISSUE-3 | MARCH - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Original Research Paper

PREVALENCE AND SUSCEPTIBILITY OF EXTENDED SPECTRUM BETA - LACTAMASES IN URINARY ISOLATES

Urology

KEYWORDS: ESBL,

Dr Bhargava Vardhan Reddy	Associate Prof, Dept of Urology, Santhiram General Hospital Nandyal.
Dr R Malleswari	Associate Prof, Dept of Anaesthesia, Santhiram General Hospital, Nandyal.
Dr Dushyanth	Junior Resident, Dept of General Surgery.

ABSTRACT

Extended spectrum beta – lactamases (ESBLs) are on the rise in hospitalsettings across the globe. The presence of ESBLs significantly affects the outcome of an infection and poses a challenge to the management of infection worldwide. Therefore, the aim of the present study is to determine the prevalence and susceptibility of extended spectrum beta – lactamase in urinary isolates in a regional hospital, Nandyal, Andhra Pradesh. A total of 300 urinary tract specimens collected from inpatients of Department of urology over a period of one year from January 2015 to January 2016.

Antimicrobial susceptibility testing was determined to commonly used antibiotics. The prevalence of ESBL was 62.5%. The ESBL producing isolates were significantly resistant ($p < 0.01$) to ampicillin, trimethoprim /sulfamethoxazole, norfloxacin and nalidixic acid as compared to non-ESBL producers. Multidrug resistance was significantly ($p < 0.01$) higher (69%) in ESBL positive isolates than non-ESBL isolates (21%). Knowledge of the prevalence of ESBL and sensitivity pattern of bacterial isolates in a geographical area will help the clinicians to formulate the guidelines for antibiotic therapy to avoid inappropriate use of extended spectrum cephalosporins.

INTRODUCTION

Resistant bacteria are emerging worldwide as a threat to favorable outcome in the treatment of common infections in community and hospital settings1. Among the wide array of antibiotics, β- lactams are the most widely used agents. The most common cause of resistance to β-lactam antibiotics is the production of β-lactamases. Over the years, many new β-lactam antibiotics have been developed; however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and over use of new antibiotics in the treatment of patients has resulted in the emergence of new variants of β-lactamase. Extended spectrum b¬lactamases (ESBLs) are defined as b-lactamases capable of hydrolyzing oxyimino cephalosporins and are inhibited by b-lactamase inhibitors.[1] The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options.[2] Microorganisms responsible for urinary tract infection (UTI) such as E.coli and Klebsiella spp. have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat.[3] There are not enough data on the prevalence of ESBL producers in urinary tract infection in regional health care centers. Hence, the present study was undertaken to find out prevalence and susceptibility of ESBL producers in urinary isolates and to study the predisposing factors for ESBL producing organisms.

Materials and Methods:

A total of 300 urine samples, over a period of one year from January 2015 to January 2016 in Department of urology, Santhiram General Hospital, Nandyal, AP. These samples were cultured and microorganisms were identified on the basis of conventional microbiological procedures7.

Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method as per CLSI recommendations8. Antimicrobial discs used were Ampicillin (10 µg), Ampicillin/Sulbactam (20/10 µg), Piperacillin-tazobactam (100/10 µg), Cephotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Amikacin (30 µg), Norfloxacin (5 µg), Nalidixic acid, Nitrofurantoin, Trimethoprim-sulfamethoxazole (1.25/23.75 µg), and Imipenem (10 µg).

E-test ESBL strips: Confirmation of ESBL was also done by E-test ESBL strips (AB BIODISK, Solana, Sweden), and the test was performed in accordance with the manufacturer's guidelines. Double ended strips containing gradient of cefotaxime or ceftazidime at one end and cefotaxime or ceftazidime plus clavulanic acid at the other end were tested. The presence of ESBL was confirmed if the ratio of the MIC of cefotaxime or ceftazidime to the MIC of cefotaxime or ceftazidime plus clavulanic acid was ≥ 8.

Results:

A total of 300 urinary isolates were collected over a period of one year from January 2015 to January 2016. By the screening test, 105of the 300 isolates were short listed as culture positive. Of the 105 isolates, 65 isolates were found to be ESBL producers by phenotypic confirmatory test. This was further confirmed by MIC using the E-test strips. A significant proportion of the ESBL producing strains were found to be resistant to antimicrobial agents including ampicillin (100%),ampicillin/sulbactam (81.29%), nalidixic acid (70.88%), piperacillin/tazobactam (51.89%),trimethoprim/sulfamethoxazole (78.48%), nitrofurantoin (74.68%), norfloxacin (51.89%) andamikacin (54.43%). Imipenem was found to be the most effective antibiotic against ESBL producers (97.53% of isolates were sensitive); while in non- ESBL producing isolates, resistance was nil. ESBL producing isolates were resistant to more antimicrobial agents than non-ESBL producing isolates. The highest rate of resistance in ESBL negative isolates was seen against ampicillin (81.29%) which was significantly ($p < 0.01$) lower than ESBL producing isolates. This was followed by resistance to ampicillin/sulbactam (78.29%). However, in this case, the difference was not significant ($p > 0.05$) (Table 1). Multidrug resistance was seen in 69.14% ESBLpositive isolates and 21.66% non-ESBL isolates. This difference was highly significant ($p < 0.01$).

Table 1: Diagnosis of the patients found to be ESBL producers

Diagnosis	Percentage %
Calculous disease	37
BPH	17
Urethralstricture	9
Acute pyelonephritis	6
Emphysematous pyelonephritis	6
VUR	5
NGB	5
PUJO	3
Carcinoma bladder	3
GU Koch's	3
Hypospadias	3
RCC	1.5
Epididymoorchitis	1.5

Risk Factors

VOLUME-6 | ISSUE-3 | MARCH - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Previous antibiotic usage was the most common risk factors associated with ESBL positive in this study, followed by previous urological intervention, diabetes mellitus, septicemia.

Table 2: Risk factors associated with ESBL producers

Risk factor	Percentage %
Previous antibiotic usage	42 (65 %)
Previous urological intervention in	27 (41.5 %)
History of diabetes mellitus	21 (32 %)
Septicemia	17 (26.1 %)

Previous intervention

Urinary catherarization and stenting are the most common procedures which are associated with ESBL in this study

Table 3: Interventions associated with ESBL producers

Previous intervention	Percentage %
Catheterization	09 (13.8%)
DJ stenting	10 (15.3%)
PCN	2 (3 %)
CIC	3 (4 %)
Serial dilatation	3 (4%)
Stage I repair of Hypospadias	1 (1.5 %)

Previous antibiotic usage

Fluroquinolones and cephalosporin's are the most common antibiotics used previously are associated with ESBL producers.

Table 4:Previous antibiotic usage

Antibiotic used	Percentage %
Fluroquinolones	20(30.7 %)
Cephalosporin's	15(23.1 %)
Cotrimoxazole	5(7.8 %)
Nitrofurontoin	2(3 %)

ESBL producing organisms Escherichia coli-73.9%, Klebsiella pneumonia -26.1%.

Antibiotics with greatest activity against ESBL producing strains were found to be Imipenem 95.3 %, Amikacin 64.6 %,Nitrofurantoin 57.0 %,Piperacillin+ Tazobactum 50.7% , Meropenem 38.4%, Cotrimoxazole 23.0% , Nofloxacin44.9%, Nalidixic acid 73.5%,Cefaperazone +sulbactum 10.7 %, GM 12 %, Flouroquinolones 06 %.

DISCUSSION:

This study demonstrates the presence of ESBL mediated resistance in urinary isolates of E.coli in Santhiram General hospital in Nandyal, AP, India. The prevalence was 60%. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes. Previous studies from India have reported ESBL production varying from 28% to 84%(9). There is considerable geographical difference in ESBLs in European countries. Within countries, hospital-to-hospital marked variability occurs 10. A large study from more than 100 European intensive care units (ICU) found that the prevalence of ESBLs in Klebsiella ranged from as low as 3% in Sweden to as high as 34% in Portugal11. In Turkey, a survey of Klebsiella sp. from ICUs from eight hospitals showed that 58% of 193 isolate harbored ESBLs12. Moland and colleagues have shown that ESBL-producing isolates were found in 75% of 24 medical centers in the United States13. ESBLs have also been documented in Israel, Saudi Arabia, and a variety of North African countries14-16. From China, the figures of ESBL producers vary between 25- 40%17. National surveys have indicated the presence of ESBLs in 5-8% of E. coli isolates from Japan, Korea, Malaysia and Singapore but 12-24%of isolates from Thailand, Taiwan, Philippines and Indonesia4.

ESBLs have emerged due to selective pressure imposed by extensive use of antimicrobials, especially in intensive care units. Since show false susceptibility to expanded-spectrum cephalosporins in standard disc diffusion test, it is essential to adopt the specific detection methods recommended by CLSI. The high rate of resistance noted among the isolates in the present study, is of serious concern. 60% of urinary isolates were ESBL producing. In this study, ESBL producing isolates were significantly more resistant to ampicillin ($p < 0.01$), nalidixicacid ($p < 0.01$), cotrimoxazole ($p < 0.01$), nitrofurantoin ($p < 0.01$), norfloxacin ($p < 0.01$) and amikacin ($p < 0.01$) as compared to non-ESBL producing gram-negative isolates.

In our study, resistance to third generation cephalosporin's was found to coexist with resistance to two or more antibiotics like ampicillin, nalidixic acid, cotrimoxazole, nitrofurantoin, norfloxacin, amikacin as also reported by Subha et al18 and Duttaroy et al19 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 plasmids20. Almost all the ESBL-positive isolates were found to be resistant to Ampicillin and sensitive to Imipenem, which again advocates the usage of carbapenem antibiotics as the therapeutic alternative to β-lactam antibiotics as indicated in many previous studies.

ESBL organisms are spread between patients through the contaminated hands , equipment and healthcare workers. The production of beta-lactamase may be of chromosomal or plasmid origin. Plasmid mediated production is often acquired and such transferable plasmid also codes for resistant determinants to other antimicrobial agents. ESBL producing organisms are resistant to all penicillins, cephalosporins and aztreonam, irrespective of routine susceptibility results.Use of 3rd generation cephalosporins is the most important factor for acquiring ESBLs. Treatment failure and deaths have occurred when cephalosporins were used against ESBL producers that appeared susceptible in vitro.(Evaluation and standards laboratory , centre for infections- 2006). The combined competences of clinicians, microbiologists and infection control team are needed to overcome these problems.

CONCLUSIONS

The prevalence of ESBL producers at our institute was 60% in accordance to the prevalence reported from other hospitals in India as well as across the globe. Multidrug resistance was significantly ($p < 0.01$) higher (69%) in ESBL positive isolates than non-ESBL isolates (21%). All the ESBL-positive isolates were found to be sensitive to Imipenem, which again advocates the usage of carbapenem antibiotics. Prior urological intervention appear to be risk factors for the presence of ESBL producing organisms. Routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI. Empirical treatment strategies and antibiotic policies may need to be re-thought in settings and locales where ESBL producers are prevalent and where there is significant perceived risk for an individual. Prevention and control measures are important because of the multi-resistant nature of these pathogens. The high level of ESBL prevalence in our set up should ensure regular monitoring and judicious usage of extended-spectrum cephalosporin's, periodic surveillance of antibiotic resistance patterns, and efforts to decrease empirical antibiotic therapy. This would go a long way in addressing some of the problems associated with ESBLs. The control measures include judicious use of antibiotics, strict hand-hygiene protocols, and implementation of appropriate infection-control measures in the hospital, especially while treating high-risk patients.

REFERENCES:

1. Chaudhary U, Aggarwal R. Extended spectrum β lactamases (ESBL): an emerging threat to clinical therapeutics. Indian J Med Microbiol.2004;22:75-80.
2. Bradford PA. Extended spectrum β lactamases in 21st century: characterization, epidemiology and detection of this important resistance threat.ClinMicrobiol Rev. 2001 Oct;14(4):933-51.
3. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum β lactamase producing nKlebsiellapneumoniae in a tertiary care hospital. Indian J Med Microbiol.2004;22:87-91.
4. Paterson DL, Bonomo RA. Extended spectrum β lactamases: A clinical update.ClinMicrobiol Rev. 2005 Oct;18(4):657-86.
5. Duttaroy B, Mehta S. Extended spectrum β lactamases (ESBL) in clinical isolates of Klebsiellapneumoniae and Escherichia coli. Indian J PatholMicrobiol.2005;48:45-8.

6. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum lactamases among enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol.2006;24:208-11.
7. Koneman EW, Allen SD, JandaWM,et al. The enterobacteriaceae.In: Color atlas and textbook of diagnostic microbiology, 5th ed. JB Lippincott Co: Philadelphia; 2006:211-302.
8. Clinical Laboratory Standards Institute; Performance standards for antimicrobial disc susceptibility testing. 14th Informational Supplement: 2004.
9. Das A, Ray P, Garg R, et al. Extended spectrum beta-lactamase production in Gram negative bacterial isolates from cases of septicemia. Proceedings of the Silver Jubilee Conference.All India Institute of Medical Sciences, New Delhi, 2001.
10. Babini GS, Livermore DM. Antimicrobial resistance amongst Klebsiella spp. Collected from intensive care units in Southern and Western Europe in 1997-1998. J AntimicrobChemother.2000 Feb;45(2):183-9.
11. Hanberger H, Garcia-Rodriguez JA, Gobernado M, et al. Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups.JAMA. 1999 Jan;281(1):67-71.
12. Gónseren F, Mamikoılu L, Oztórk S, et al. A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. AntimicrobChemother.1999Mar;43(3):373-8.