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Isolation of Extended Spectrum B-Lactamases in Urine Samples

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ABSTRACT

Background*: Extended spectrum β-lactamase (ESBL) production among uropathogens is an important marker of endemicity.*

Materials and Methods: *During the study period of 7 months, 960 urine samples were processed for significant bacteriuria.*

Results: *Out of these 960 Urine samples 611 (63.3%) showed significant growth. Among these E.coli and klebsiella species has highest prevalence of ESBL. Maximum number of ESBL producers were seen in the age group of 40 – 60 years. Females show higher percentage of ESBL producers compared to males.*

Conclusion: *This study reveals the prevalence of ESBL producing organisms is a significant uropathogen in our area. Constant revision of antibiotic policies with infection control interventions is suggested.*

Keywords: *ESBL, Endemicity, Infection, Uropathogens.*

INTRODUCTION

Extended spectrum β- lactam antibiotics such as third generation cephalosporins form the major component of empiric antibacterial therapy in most clinical setups and especially in tertiary care centers, extensive use of 3rd generation cephalosporins has contributed to evolution of extended spectrum β-lactamase (ESBL) producing organisms. These isolates were first detected in western Europe in mid 1980s since then their incidence has been increasing steadily. A large number of out breaks of infections due to ESBL producing organisms have been described in every continent of the globe. In some hospitals, initial outbreak of infections have been supplanted by endemicity of the ESBL producing organisms. This may lead to increased patient mortality when antibiotics are inactivated by ESBL producers. Therefore, control of the initial outbreak of ESBL producing organisms in a hospital or specialized unit of a hospital is of critical importance.[1]

Special efforts should be undertaken by clinical microbiology laboratories as recommended by clinical and laboratory standards institute (CLSI) for ESBL detection. Additional use of ESBL detection methods has originated because of some ESBL producing organisms appeared susceptible to cephalosporins, using conventional breakpoints. It has been recommended that physicians should avoid all penicillins, aztreonam and cephalosporins if an ESBL producing organism is present.

β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics in gram negative bacteria. In recent years there has been an increased incidence and prevalence of extended spectrum β-lactamases (ESBLs), enzymes that hydrolyze and cause resistance to oxymino-cephalosporins and aztreonam. ESBLs represent a major group of β-lactamases currently being identified worldwide in large numbers and are now found in a significant percentage in E.coli and K.pneumoniae strains. They have also been found in Pseudomonas aeruginosa and other Enterobacteriacae strains like Enterobacter, Citrobacter, Proteus, Morganella morganii, Serratia marsescens, Shigella dysenteriae, Burkholderia cepacia and Capnocytophaga ochracea.

These organisms are now been also isolated from normal human feacal sample i.e. these organisms now became the normal commensal flora of the intestine and causing community acquired drug resistant infection thus strict infection control including biomedical waste management, avoiding over the counter usage of antibiotic and judicial use of antibiotics by the physicians . Hence we aim in studying prevalence of ESBL producing uropathogens in symptomatic urinary tract infections in patients.

MATERIALS AND METHODS

It is a study done from may 2014 to november 2014 including 960 in the Department of Microbiology with the samples obtained from OP in symptomatic urinary tract infection. Urine samples received in the microbiology laboratory were processed following standard protocol. All the gram negative bacilli isolated in significant numbers were identified by standard microbiological procedures and sensitivity including third generation cephalosporins (3GC, ceftazidime, ceftriaxone, and cefotaxime) was determined in Urichrome agar media, Mueller Hinton agar media[MHA] media. Results were interpreted according to CLSI guidelines.

Preparation of inoculums

Midstream urine sample was collected from patients clinically suspected to be suffering from Symptomatic urinary tract infection.

Urine was cultured in Urichrome agar medium and colonies are detected after aerobic incubation.

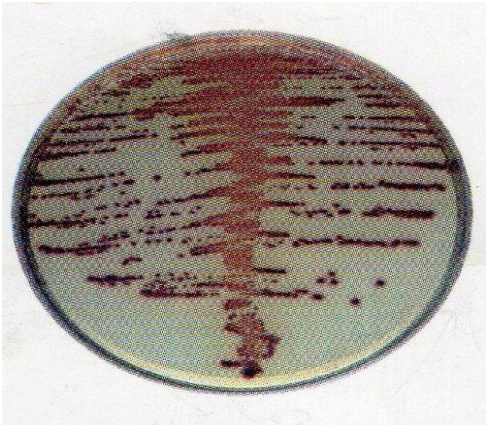


Fig1: E.Coli colonies

Fig2: Antibiotic sensitivity zones

Antimicrobial susceptibility testing was performed as per guidelines.

Emulsify the colonies in 2ml of peptone water.

The test inoculums [0.5Mc farland turbidity]was spread onto muellere Hinton agar[MHA]using a sterile cotton swabs. A Disc of Amoxicillin with Clavulanic Acid (AMC) and Ceftazidime (CAZ) are arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone.

The plate was incubated over night at 37°C. After aerobic incubation antibiotic sensitivity zones were measured according to CLSI guidelines.

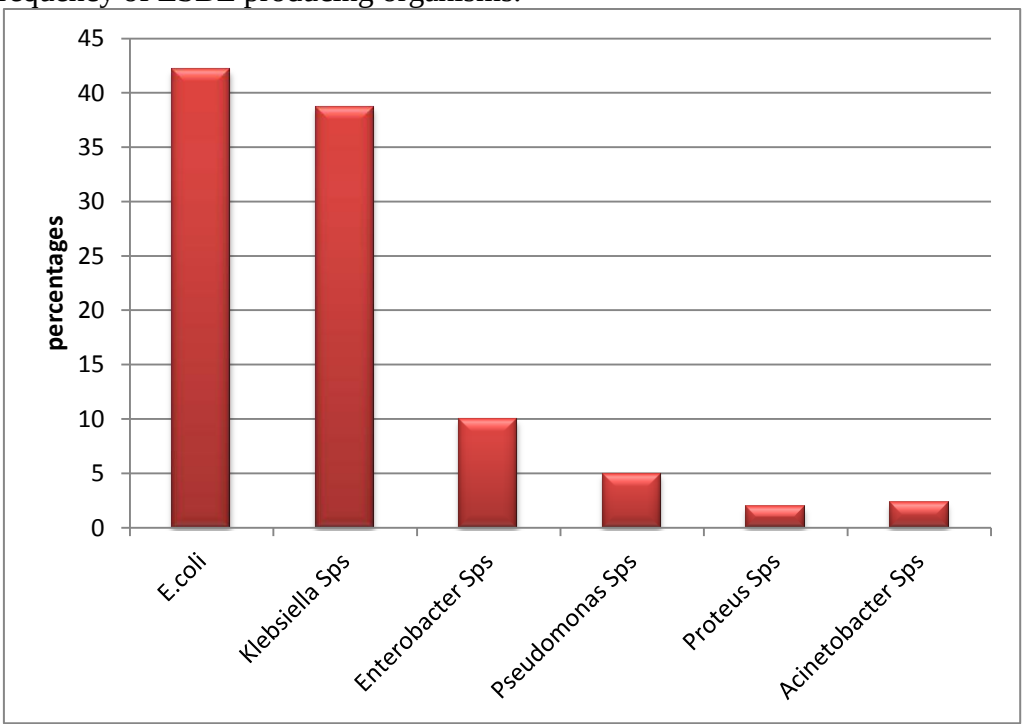
RESULTS

A total of 960 urine samples from patients were collected during 7 months study period. Total number of positive isolates 611 and ESBL producers are 261.

Table1: Distribution of ESBL in isolated uropathogens

Clinical isolates	Positive Isolates	ESBL Isolates	Percentage
E.coli	352	110	42.14
Klebsiella Species	175	101	38.69
Enterobacter Species	34	26	9.96
Pseudomonas Species	17	13	4.98
Proteus Species	26	05	1.91
Acinetobacter Species	07	06	2.29
Total	611	261	100

Figure-3: Frequency of ESBL producing organisms.



Out of these 960 Urine samples 611 (63.3%) showed significant growth.
Among these E.coli and klebsiella sps has highest percentage (%) of ESBL.

Table-2: Age wise distribution of ESBL producing organisms.

Age intervals (in yrs)	Gram –ve Bacillus	ESBL	Percentage
0-9	0	0	0
10-19	35	8	3.07
20-29	32	19	7.28
30-39	88	43	16.47
40-49	109	35	13.41
50-59	166	55	29.07
60 Above	181	101	38.69
Total	611	261	100

Maximum number of ESBL producers were seen in the age group of 40 – 60 years.

High prevalence of UTI in old age (60 above) group male may be due to different conditions like Prostatis, Diabetes and weak immune status.

Table-3: Distributionof ESBL in male and female patients.

Sex	Gram –ve Bacillus	ESBL	Percentage
Male	226	72	27.58
Female	385	189	72.42

Females show higher percentage of ESBL producers compared to males.

DISCUSSION

Since there appearance in Germany in early 1980's ESBL producing bacteria have increase number [2]. Early there were isolated from hospitalized patients only but now reports of isolation from outdoor patients have started coming [3,4]. Antibiotic resistance surveillence has a central role among all strategies to manage the problem of antibiotic resistance. [5] In present study of the detection rates of ESBL producing isolates gram negative organism was 42.7% which is co-relate with following articles. The study of Tankhi wale et al[6] detected 46.3% urinary isolates to be ESBL producers E.coli, Klebsiella and Acinetobacter species being predominant. In study from south India 41% of E.coli and 40% of Klebsiella are found to be ESBL produces in isolates of patient suffering from urinary tract infection.[7]

Studies [8] shows 32% E.coli, 37%, Klebsiella species and 20%, citrobacter species among the urinary pathogens produces ESBL. Akram et al [9] detected 34.42% of ESBL produces among E.coli in urinary isolates. Ritu agarwal et al[10] detected 36% urinary isolates produced ESBL. [1] C. Rodriguez et al showed 53% were ESBL producers. Urinary tract infection is found to be more prevalent in females than males. Wong et al[11] in the present study ESBL isolates detected in males were (38.7%) 196 and in females 310(61.2%).

In previous studies, prolonged hospitalization, Foley's catheterization, prior surgery, and ICU stay were found to be risk factors. Good infection control practices and antibiotic management interventions are instrumental in preventing the emergence of outbreaks due to ESBL producing isolates, especially in high risk areas such as the medical ICU, the neonatal ICU, and oncology units. Educational programs for medical staff to increase awareness of ESBLs should also be developed.

Our study confirms the global trend toward increased resistance to β-lactam antibiotics. Prevalence and antibiotic susceptibility pattern of ESBL producers differs geographically. As a global trend towards increased resistance to beta-lactum antibiotics .It is emphasize that institutions with high prevalence of 3rd generation cephalosporins resistant organism should employ

appropriate antimicrobial Steward ship to avoid indiscriminate use of 3rd generation cephalosporins and also institutes must formulate antibiotic policy[12]. Basing on the organism sensitivity pattern and prescribing appropriate antibiotics will reduce the patient morbidity and motality and contributes to decrease health care cost. Hence, such studies will help in the formulation of antibiotic policy for a particular geographical area.

CONCLUSION

The resistance to antibacterial agent is a major public health problem. The present study provided information of prevalence of antimicrobial resistance among pathogens causing urinary tract infections rising antibiotic resistance among urinary isolates emphasized. The importance of some hospital infection control rational prescribing policies and need of new antimicrobial drugs.

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Conflict of Interest: None declared.

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REFERENCES

1. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1989;33:1131–6.
2. Nathisuwan S, Burgess DS, Lewis JS., 2nd Extended Spectrum β-lactamases: Epidemiology, Detection and treatment. Pharmacotherapy. 2001;21:920–8.
3. Ananthkrishnan AN, Kanungo R, Kumar A, Badrinath S. Detection of extended spectrum beta lactamase producers among surgical wound infections and burn patients in JIPMER. Ind J Med Microbiol. 2000;18:160–5.
4. Brigante G, Luzzaro F, Perilli M, Lombardi G, Colì A, Rossolini GM, et al. Evolution of CTX-M type beta - lactamasees in isolates of Escherichia coli infecting hospital and community patients. Int J Antimicrob Agents. 2005;25:157–62.
5. Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian J Med Microbiol. 2002;20:92–5.
6. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U.Evalutaion of extended spectrum beta lactamase in Urinary Isolates. Indian J Med Res 2004;120:553-6
7. Hasan Ejaz1, Ikram-ul-Haq1, Aizza Zafar, Saqib Mahmood:Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniaeAfrican Journal of Biotechnology 2011:10(73),16661-16666,
8. T.Menon, D Bindu, CPG Kumar, S Nalini, MA Thirunarayan, Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. Indian Journal of Medical Microbiology, 2006;24(2):117-120.
9. Akram M, Shahid M, Khan AU, Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital, Aligarh, India. Ann Clin Microbiol Antimicrob 2007;6:4
10. Ritu Agarwal, Uma Chowdhary, Rama Sikka Indian Journal Medical,Microbiology (2004; 2:75-80)
11. Wong ES, Stamm WE. Sexual acquisition of urinary tract infection in man. J Am Med Assoc 1983;250:3087-8
12. C Rodrigues, P Joshi, SH Jani, M Alphonse, R Radhakrishnan, A Mehta: Indian Journal of Medical Microbiology, (2004) 22 (4): 247-250