EVALUATION OF EXTENDED SPECTRUM BETA – LACTAMASE MEDIATED RESISTANCE IN ESCHERICHIA COLI AND KLEBSIELLA IN URINARY TRACT INFECTION AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Urinary tract infections (UTIs) caused by extended – spectrum beta lactamase (ESBL) – producing bacteria have become a growing problem due to emerging antibiotic resistance. This observational study was carried out from January 2012 to August 2012 to see the frequency and antibiotic susceptibility of ESBL producers E.coli and KI.pneumoniae in urinary tract infection.

Materials and Methods: A total of 672 urine sample received at Microbiology laboratory of Shalamar Medical College, Lahore and processed for culture / sensitivity.

Results: On culture 256 isolates were obtained, of which 163 isolates were identified by $AP_{120}E$ as E.coli 125 (76.3%) and K.pneumoniae 38 (23.3%). The ESBL producing E.coli 90 (72%) and Klebsiella pneumoniae 25 (65.8%) were detected by double disc synergy test (DDST). Sensitivity testing showed a multidrug resistance in ESBL producing E.coli and K.pneumoniae. Maximum resistance recorded in E.coli (ESBL) was as cafatoxime (98.9%), Ceftazidime (96.7%) and Cefuroxime (93.4%) while minimum resistance was seen with Imipenum (0.8%) fosfomycine (1.2%) and Nitrofurantoin as well piperacillin / tazobactam (2.2%) each. The ESBL producing Klebsiella showed maximum resistance tocetoceftazidime (100%) cefotaxime (89%) Cefuroxime (84%) while minimum resistance seen with imipenam (4%) Nitrofurantoin and Piperacillin / Tazobactam (8%).

Conclusion: ESBL producing bacteria, area matter of concern in high frequency of antimicrobial resistance to non beta lactam and aminoglycocides antibiotics. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

INTRODUCTION

Urinary tract infections (UTIs) in healthy adults are usually treated empirically because the causative microbe is highly predictable: 80% – 90% are caused by Escherichia coli. (Behzadi et al; 2010, Moammed et al; 2007). In addition, short courses of therapy (1 day or 3 days) are usually completed before laboratory results become available. (Pai and Nai; 2012, Biswas et al; 2006).

Microorganisms responsible for urinary tract infection (UTI) such as E.coli, Klebsiella, Citrobacter species and others Gram negative pathogens harboring Extended Spectrum Beta Lactamases (ESBLs) destroy cephalosporins and making them very difficult to eliminate because of their multi-drug resistance to various classes of antibiotics such as cephalosporins monobactam, carbapenems, ciprofloxacin, and erythromycin and β-lactamase inhibitor combination. ESBL – mediated resistance is not always obvious in vitro to all cephalosporin Many ESBL producers are multi-resistant to non-β-Lactam antibiotics such as quinolones and amino glycosides, narrowing treatment options. (Jalapour; 2012). ESBL resistance genes are encoded on freely transmissible genetic elements, greatly increasing the risk of spread to other organisms. ESBLs are plasmid mediated transferrable enzymes capable to hydrolyze third and fourth – generation cephalosporins and monobactams which may be inhibited by clavulanic acid (Tribuddharat, et al; 2007). Unlike MRSA or VRE, the resistance mechanisms of ESBLs are not limited to one or even two species but rather a whole family of organism, the Enterobacteriaceae. Enterobacteriaceae has become one of the most important causes of nosocomial and community – acquired infections. The main therapeutic choices to treat such infections are β-lactam antibiotics (mainly broad spectrum penicillins and cephalosporins). These antibiotics have a common element in their molecular structure: a four – atom ring known as a beta-lactam. The lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties. The ability to produce ESBLs in large quantities (Anwar et al; 2007). These enzymes are plasmid – borne and confer multiple drug resistance, making UTI difficult to treat (Gracia et al; 2007).

MATERIALS AND METHODS

Urine samples were received in Microbiology Department of Shalamar Medical and Dental College, La-

hore; from patients attending the Outpatient Department and admitted in the wards at Shalamar Hospital from Jan 2012 to Aug 2012, the study samples included midstream and catherized urine. All samples were inoculated on CLED agar and incubated at 37°C for 24 hours, and for 48 hours in negative cases. A pure, significant (> 105 cfu) bacterial growth of E.coli and K.pneumoniae were identified by $API_{20}E$. These isolates were tested for antimicrobial susceptibility by Kirby – Bauer disc diffusion technique using Muller Hinton agar. The antibiotic discs used were Cotrimoxazole (25 µg),Ampicillin (10 µg), Amikacin (30 µg), Gentamicin (20 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Nitrofurontoin (300 µg), Fosfomycin (200 µg) Cepfoerazone / Sulbactam (75/30 µg), Piperaacillin / Tazobactam (110).

Susceptibility test results were recorded according to clinical laboratory standard institute (CLSI). Screening test for ESBL was done according to the criteria recommended by CLSI an inhibition zone of ≤ 27 mm for cefotaxime and ≤ 27 mm for cefotaxime and ≤ 22 mm for ceftazidime indicated that the strain probably produced ESBL. Phenotypic confirmatory test for ESBL was performed using double disk synergy Test (Jabeen, et al 2003). Muller Hinton agar plates was swabbed with the standard inoculums (corresponding to 0.5 McFarlands standard) to form a lawn culture. A susceptibility disc containing Amoxicillin – acid disc (20/10 µg) was placed in the center of the plate and a disc of Cefotaxime (30 µg) and the Cefrazidime (30 µg) was placed 20 mm apart, from Amoxicillin – Clavulanic acid disc. Plates were examined for enhancement of zone inhibition of Cefotaxime and Feftazidime respectively. Organisms that showed a clear extension of inhibition zone towards the disc Augmentin were considered ESBL positive.

RESULTS

A total of 672 urine sample were received for culture / sensitivity in microbiology department of shalamar Medical and Dental College, Lahore during the time period of eight months (January 2012 to Aug 2012) from children and adults. The total of 256 growths was obtained which included 163 E.coli and Klebsiella and 83 other uropathogens. Of 163 isolates, 125 (76.6%) were E. coli and 38 (23.4%) were Klebsiella pneumoniae identified by the use of $API_{20}E$. In this study total ESBL detected were 103 (63%) of all the isolates. Amongst these ESBL – producing E. coli strains were 90 (72%) while K.pneumoniae 25 (65%). Non ESBL – producing E. coli were 35 (28%) and K.pneumonia were 13 (34.2%) (Fig. 1).

DISCUSSION

Urinary tract infections (UTI) are the most common infections diagnosed in outpatient as well as hospitalized patients. The microorganisms in this study are E.coli (125/163 – 76.3%) and Klebsiella pneumoniae (38/163 – 23.3%) the most commonly isolated pathogen in urinary tract infection. Out of which ESBL producer E.coli were 90/125 (72%) and Klebsiella pneumonia 25/38 (65.8%). This study was suppor-

Table 1: *Antibiotic sensitivity pattern of ESBL – producers and non-ESBL producers.*

	AK	*CN*	*SXT*	*CIP*	*NA*	*F*	*IPM*	*CTX*	*CAZ*	*CRO*
E.coli (125)										
ESBL producers (90)	24 (26.6%)	50 (55.5%)	71 (79%)	70 (77.8%)	46 (50%)	02 (2.2%)	00 (0%)	98 (98.9%)	87 (96.7%)	84 (93.4%)
Non-ESBL producers (35)	10 (28.5%)	18 (51.4%)	25 (71.1%)	24 (68.4%)	15 (42.8%)	18 (51.4%)	01 (5.7%)	02 (5.7%)	03 (8.7%)	01 (2.5%)
Klebsiella species (38)										
ESBL producers (25)	03 (12%)	23 (72.2%)	15 (48.5%)	07 (30.7%)	07 (30.7%)	02 (8%)	01 (4%)	23 (81.2%)	25 (100%)	21 (80.1%)
Non-ESBL – (13) producers	02 (15.4%	21 (84%)	05 (38.4%)	03 (26.2%)	01 (7.6%)	02 (15%)	01 (7.6%)	01 (7.7%)	02 (15.3%)	02 (15.3%)

AK: amikicin, CN: gentamicin, SXT: Co. trimoxazole, CIP: ciprofloxacin, NA: naladixic acid, F. nitrofurontoin, IPM: immipenam, CTX: ceftoxime, CAZ: ceftazimide, CRO: cefuroxime

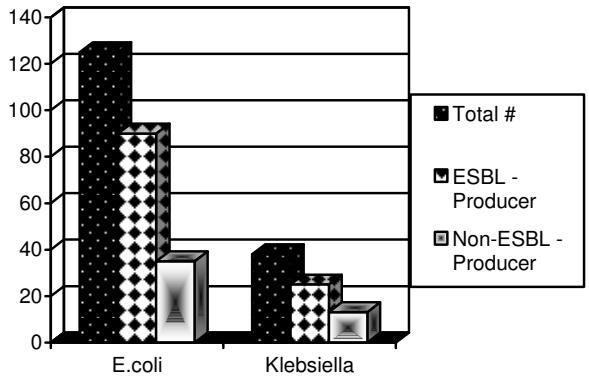

Fig. 1: *Frequency of ESBL and non-ESBL producing bacteria in urinary tract infection.*

ted by the other studies where E.coli and Klebsiella were the major pathogen of urinary tract infection (Hummyun and Iqbal; 2010, Mayo et al; 2010, Qureshi, 2005 and Ejaz et al; 2011).

The prevalence of ESBL producing E.coli and Klebsiella is increasing rapidly in the past few years. This creates an alarming situation because prevalence of ESBL, type of enzyme, gender and age group in which ESBL is present varies in different geographical area.

In the current study the ESBL producing E.coli and Klebsiella (72% and 65.8%) have emerged as multidrug resistant pathogen both in hospital and community acquired infections. Other studies revealed significant high rate inpatient infected with ESBL producing organism. (Mahrgan and Rahbar; 2008). While (Coqu et al; 2008 and Khanfar et al; 2009) reported most commonly community acquired infections. Our study showing that male of older age and female of young age were more prone of infection caused by ESBL producers, similarly reports documented by (Riaz et al; 2011 and Khanfer; 2009) ESBL – producing E. coli significantly infreased from 2 (0.20%) to 89 (5.52%) isolates per year. In this study the ESBL producing E.coli and Klebsiella were 72% and 51.5% respectively. Prevalence of ESBL was more among E.coli. These results were found slightly higher than study carried out in India (Shobha et al; 2007, Abilash et al 2010) where the ESBL positive E.coli 57.4% and Klebsiella 71.7%. The only differrence is in the present study, E.coli were higher than ESBL producing klebsiella. A study conducted in Iran (Behrozzi et al; 2010). Prevalence of E. coli was (21%) and 18 (12%) K. pneumoniae (Taneja et al; 2008) reported 40.2%) E. coli isolates and 51.2% of K. pneumoniae ESBL producers. Antimicrobial resistance to ESBL producing E.coli and Klebsiella in this study is recognized as multidrug resistance. Both organism showed highest resistance to Ceftriaxone, ceftoxame and minimum resistance was observed by carbepanems drugs. In some other similar studies. (Maratani et al; 2006, Lindback et al 2010). Frequency of drug resistance in ESBL producer. (Bashir et al 2008) recorded high to commonly used drug in Urinary tract infection. In this study immipenum, Nitrofurontoin Amikacin showed high susceptibility Mekki et al (2010) reported high resistance of naladixic acid, nitrofurontoin, contrimoxazole, gentamicin 100% for each, Ciprofloxacin 97.9% and Amikacin 69.4%. In a study carried out in Bungladesh (Alipourfard et al 2010) reported 93.9% Amiakcin, 57.4% Nitrofurointoin. 100% resistance to (1 – 4 gen) cephalosporin 74% resistance to all quinolons Ampicillin. Co-trimoxazole. Ampicillin + claulanic acid showed 100% resistance. Another study by Jalalpou (2012) described resistance to Co Trimoxaole 52.2%, Naladixic 54.9% Ciprofloxacin 30.3% Gentamicin 27% and 16% nitrofurontoin while Klebsiella resistance was highest with Amakacin 75% and least with nitrofurontoin 37.5%. Most of these results are in accordance with our study except immipenum, fosfomycin, nitrofurantoin where susceptibility is high in our study.

Although the uropathogen profile of the present study resembles to similar studies worldwide, (Abhilash, et al; 2010, Hassan et al; 2007, Ullah et al; 2009) the antibiotic resistance of these organisms was unusually high. Cotrimoxazole is the recommended drug for treating UTI. However, more than one third of the study subjects were resistant to the first–line drug. 76% the uropathogens were resistant to fluoroquinolones, which are considered as the second – line drug.

It is ***concluded*** that high prevalence of ESBL – producing E. coli and Klebsiella spp strains was found both among out and inpatients. Most of the ESBL – producing isolates were multi-drug resistant making available therapeutic choices limited. We recommend continued antibiotic surveillance as well comprehensive multi-center studies to address the emerging problem of ESBL – associated infections in order to preserve the continued usefulness of most antimicrobial drugs. Furthermore conducting molecular studies will help to evaluate the various ESBL types.

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