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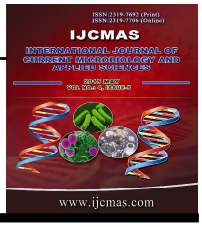

Original Research Article

Urinary Isolates with Special Reference to ESBL Producers

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A B S T R A C T

Uropathogens have shown a slow but steady increase of resistance to several antimicrobials over the last several decades. Extended Spectrum Beta Lactamases (ESBL) hydrolyse the expanded spectrum cephalosporins, used in the treatment of Urinary Tract Infections (UTIs). A total of 962 urine samples were processed on Urichrom II agar, and the plates showing significant colony count were further processed for identification and sensitivity (including ESBL detection). Out of the 962 urine samples processed, 416 samples were positive (43.24%) for significant bacterial growth. Of them Gram positive isolates were obtained from 54 samples (12.96%) and Gram negative isolates from 362 samples (86.93%). *Escherichia coli* was the commonest isolate in the present study- 44.71%, followed by *Klebsiella* species- 11.29%. Among the Gram positive cocci, *Enterococcus* species was the predominant isolate- 9.6%. ESBL production in the present study was found to be 42.79%. The present study reinforces the need for the establishment of guidelines for early phenotypic detection of ESBLs in Microbiology laboratories, and for the appropriate use of antibiotics.

Keywords

Urine, significant bacteriuria, Gram negative bacilli, Gram positive cocci, ESBL

Introduction

Urinary Tract Infections (UTIs) are the second most common cause of infectious diseases in human beings, Gram Negative Bacilli (GNBs) being the most frequently implicated pathogens (1). Uropathogens have shown a slow but steady increase of resistance to several agents over the last several decades. They have become less susceptible to commonly used antimicrobials such as trimethoprim/ sulfamethoxazole and fluoroquinolones (2). It is well known that the mechanism of antimicrobial resistance could happen by enzymatic inactivation, altered receptors or by altered antibiotic transport. Extended Spectrum Beta Lactamases (ESBL) hydrolyze the expanded spectrum cephalosporins, which are used in the treatment of UTI. Some ESBLs confer high level resistance to all oxyimino beta lactams, but for other ESBLs resistance is only slightly increased, or increased selectively for particular beta lactams. This creates a problem for the clinical laboratory, since

organisms producing less active ESBLs can fail to reach the current Clinical Laboratory Standards Institute (CLSI) break points for resistance interpretation, but can cause significant disease (2). Current knowledge on antimicrobial susceptibility pattern of uropathogens is mandatory for appropriate therapy. The aim of the present study was to evaluate the emergence of ESBLs among Gram negative bacilli causing UTIs, since the failure to treat such drug resistant forms will increase the rate of morbidity and mortality in the patients.

Materials and Methods

A total of 962 urine samples were processed on Urichrom II agar, a chromogenic medium from ELITECH MICROBIO, France. The media was prepared in house according to the manufacturer's instructions. The autoclaved media were poured into petri dishes, checked for sterility, and stored at 2-8°C till used (approximately 1 week to 10 days). Each fresh batch of medium was tested by inoculating standard strains of *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Urine samples were collected in sodium borate tubes for culture. Well mixed uncentrifuged samples were inoculated onto Urichrom II agar with a standard pre-calibrated loop (Nichrome-SWG 24, 4 mm internal diameter holding 10 µl of the urine sample). The plates were incubated over night at 37^0C after inoculation, and the plates showing significant colony count were further processed for identification and sensitivity. Final identification of the organisms and interpretation of sensitivities was done using MicroScan autoSCAN-4 instrument (SIEMENS). ESBL producers were defined as organisms showing raised Minimum Inhibitory Concentrations (MICs >2µg/ml) for ceftriaxone and ceftazidime in the screening test, and if there was ≥3 two-fold dilution drop (a three well decrease) in the MIC value for the antibiotic with clavulanic acid combination in the confirmatory test (3). A positive test (≥3 two-fold dilution drop in MIC) with either of the antibiotic combination was considered ESBL phenotype confirmation positive. For all confirmed ESBL producing strains the test interpretation was reported as resistant to all penicillins, cephalosporins and aztreonam.

Results and Discussion

Out of the 962 urine samples processed, 416 samples were positive (43.24%) for significant bacterial growth, and 546 samples (56.75%) were either negative for bacterial growth or did not show significant bacterial growth. None of the samples showed mixed bacterial growth. Among the positive samples (416), Gram positive isolates were obtained from 54 samples (12.96%) and Gram negative isolates from 362 samples (86.93%).

Among the Gram Negative Bacilli glucose fermenters were isolated from 236 samples and glucose non-fermenters from 26 samples. ESBL producers were obtained from 101 (42.79%) glucose fermenters. None of the *Pseudomonas* species isolated (n=16; 3.84%) was an ESBL producer.

ß-lactamases are the collective name of enzymes that open the ß-lactam ring by adding a water molecule to the common ß-lactam bond, and this inactivates the ß-lactam antibiotics (penicillins to carbapenems) (4). ESBLs are defined as ß-lactamases that have the following characteristics: they are transferable; they can hydrolyze penicillins, first-, second-, and third generation cephalosporins, and aztreonam (but not the cephamycins); they

can be blocked *in vitro* by ß-lactamase inhibitors such as clavulanic acid (4). The Ambler molecular classification and the Bush-Jacoby-Medeiros functional classification are the two most commonly used classification systems for β-lactamases. Ambler scheme divides β-lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and not phenotypic characteristics. In the Ambler classification scheme, β-lactamases of classes A, C and D are serine β-lactamases. In contrast, the class B enzymes are metallo-β-lactamases. With the exception of OXA-type enzymes (which are class D enzymes), the ESBLs are of molecular class A (5). The Bush-Jacoby-Medeiros classification scheme groups β-lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system.

This classification scheme is of much more immediate relevance to the physician or the microbiologist in a diagnostic laboratory because it considers β-lactamase and β-lactam substrates that are clinically relevant. In this classification, ESBLs belong to group 2be or group 2d (OXA-type), the latter sharing most of the fundamental properties of group 2be enzymes though differing in being inhibitor resistant (5). Inhibition by β-lactamase inhibitors such as clavulanic acid and inability to hydrolyze cephamycins differentiates the ESBLs from the AmpC-type β-lactamases (group 1), which have third-generation cephalosporins as their substrates but which are not inhibited by clavulanic acid (5).

Most ESBLs can be divided into three groups, which are designated the TEM (approx. 200 variants), SHV (over 140 variants), and CTX-M (approx. 130 variants) enzymes (4). The CLSI has proposed dilution methods to screen for ESBL production by *Klebsiellae pneumoniae* and *K oxytoca, Escherichia coli and Proteus mirabilis*. Ceftazidime, aztreonam, cefotaxime or ceftriaxone can be used at a screening concentration of 1 μg/mL or cefpodoxime at a concentration of 1 μg/mL for *Proteus mirabilis*; or 4 μg/mL, for the others. Growth at or above this screening antibiotic concentration is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (5). Phenotypic confirmatory testing can be performed by broth microdilution assays using ceftazidime (0.25-128 μg/mL) and ceftazidime plus clavulanic acid (0.25/4 - 128/4 μg/mL); cefotaxime (0.25-64 μg/mL), and cefotaxime plus clavulanic acid (0.25/4 - 64/4 μg/mL).

Broth microdilution is performed using standard methods. Phenotypic confirmation is considered as ≥3 twofold serial-dilution decreases in minimum inhibitory concentration (MIC) of either of the cephalosporins in the presence of clavulanic acid compared to its MIC when tested alone (5). According to CLSI guidelines, isolates which have a positive phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephamycins- cefoxitin and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin.

Penicillins (for example, piperacillin or ticarcillin) are reported as resistant regardless of MIC, but β-lactam/ β-lactamase inhibitor combinations (for example, ticarcillin-clavulanate or piperacillin-tazobactam) are reported as susceptible if MICs or zone diameters are within the appropriate range (5). Since ESBL production is usually plasmid

mediated, it is possible for one specimen to contain both ESBL-producing and non–ESBL-producing cells of the same species. This suggests that for optimal detection, several colonies must be tested from a primary culture plate (5). Urine culture positivity in the present study was 43.24%, of which Gram Negative Bacilli (GNB) constituted 86.93% and Gram Positive Cocci (GPC) constituted 12.96%. Other similar reported studies of urine culture positivity include Illamani V et al (6)- 49.39%, Neelam Taneja et al (7)- 21.8%, Supriya S et al (8)- 12.06%, Dr IM Rejitha et al (9)- 28%, Hasan Ejaz et al (10)- 14.29%. The present study (43.24%) correlates well with Illamani V et al (6) - 49.39%.

The high culture positivity in the present study (normal around 20%) could be because the source of the samples was from a camp conducted at a place where there were a cluster of cases suspecting urinary tract infections clinically. *Escherichia coli* was the commonest isolate in the present study- 44.71%, followed by *Klebsiella* species- 11.29%. Among the Gram Positive Cocci, *Enterococcus* species was the predominant isolate- 9.6% in the present study. Supriya S et al (8) had reported isolation of *E coli* in 49.8% and Klebsiella species in 37.8% of their isolates. Meltem Isikgoz Tasbakan et al (11) had reported *E coli* in 45.5% and *Klebsiella* species in 13.3%. Akram Hassan Mekki et al (2) had reported *E coli* in 49% and *Klebsiella* species in 51% of their isolates. Dr IM Rejitha et al (9) had reported more common isolation of *Klebsiella* species (42.85%) in their study when compared to *Escherichia coli*- 14.28%. ESBL production in the present study was found to be 42.79%. Other reported similar studies for ESBL production include- Akram Hassan Mekki et al (2)- 53%, Enas Sh. Khater and Hammouda W. Sherifl (12)- 53.3%, Fereshteh Javadian et al (13)- 44.44%, Neelam Taneja et al (7)- 36.5%, Supriya S et al (8)- 48.3%, L. F. Nimri and B. A. Azaizeh (14)- 50.3%. Some of the authors had reported high ESBL isolation percentages in their studies- Dr IM Rejitha et al (9) - 84.6% ESBL positive, Hasan Ejaz et al (10) had reported 71.7% ESBL positive in their *Klebsiella* species isolates and Meltem Isikgoz Tasbakan et al (11)- 61% ESBL positivity. Illamani V et al (6) had reported low ESBL isolation percentage (11.6%) in their study. Babak Pourakbari et al (15) had reported 32% ESBL positivity in their isolates from samples from community acquired UTIs and 42% ESBL positivity in their isolates from samples from hospital acquired UTIs. Shahanara Begum et al (16) had reported 37.8% of their *Pseudomonas aeruginosa* isolates to be ESBL producers, while all the *Pseudomonas* isolates in the present study (n=16; 3.84%) were negative for ESBL production. ESBL production for the individual organisms in the present study was as follows- *E coli*- 45.16%, *Citrobacter* species- 23.8%, *Klebsiella* species- 17.02%, *Proteus* species- 14.28% and *Enterobacter* species- 12%. In a similar study reported by Meltem Isikgoz Tasbakan et al (11), the ESBL production in their isolates reported was *E coli*- 45.5%, *Klebsiella* species- 13.3%, *Enterobacter* species- 10.2% and *Pseudomonas* species- 10%. ESBL production for *E coli* in the present study was 45.16%. Other similar reported studies for ESBL production in *E coli* were as follows: Dinesh Kumar et al (17) - 55.55% in *E coli*, Rohini P et al (18)- 58% in *E coli*, Farhat Ullah et al (19)- 56.9% in *E coli*, Rajan S et al (20)- 34.8% in *E coli* and Hasan Ejaz et al (10)- 57.4% in *E coli*. Some authors had reported low ESBL isolation percentage in their *E coli* isolates like Datta P et al (21) - 21.4% ESBL positive for *E coli*, Jafar Mobaleghi et al (22)- 19.02% ESBL *E coli* positive.

Table.1 Organisms isolated and their percentages

ISOLATE	NUMBER (n)	PERCENTAGE (%)
GRAM NEGATIVE BACILLI		
Escherichia coli	186	44.71
Klebsiella species	47	11.29
Enterobacter species	25	6.00
Citrobacter species	21	5.04
Pseudomonas species	16	3.84
Serratia species	14	3.36
Kluyvera species	12	2.88
Proteus species	7	1.68
Rauotella ornitholytica	7	1.68
Morganella morganii	6	1.44
Cedecea species	4	0.96
Acinetobacter lwoffii	4	0.96
Achromobacter xylosoxidans	3	0.73
Yersinia species	3	0.72
Acinetobacter baumannii	2	0.48
Pantoea agglomerans group	1	0.24
Providencia rettgeri	1	0.24
Aeromonas hydrophilia	1	0.24
Ralstonia paucula	1	0.24
Hafnia alvei	1	0.24
Total gram negatives	362	86.93
GRAM POSITIVE COCCI		
Enterococcus species	40	9.6
Coagulase negative Staphylococci	10	2.4
Staphylococcus aureus	2	0.48
Streptococcus agalactiae	1	0.24
Streptococcus bovis	1	0.24
Total gram positives	54	12.96
Grand total	416	99.89

Table.2 Percentage of ESBL producers in the individual organisms

Isolate	Total Number isolated	% of ESBL producers
Escherichia coli	84	45.16
Citrobacter species	5	23.80
Klebsiella species	8	17.02
Proteus species	1	14.28
Enterobacter species	3	12.00

The increasing frequency of ESBL phenotypes is an emerging problem; and hospitalization, previous bacterial infection, urinary abnormalities, previous antimicrobial treatment (especially third-generation cephalosporins), recurrent tract infections, and the presence of high-level and multidrug resistance should be considered seriously (15). To solve the problem of ESBL-producing Enterobacteriaceae and other types of resistant bacteria, prevention is crucial and surveillance of antimicrobial resistance is needed to guide preventive interventions. Because of globalization, including international travel, it is important to have a global approach to antibiotic resistance. In conclusion the present study reinforces the need for the establishment of guidelines for early phenotypic detection of ESBLs in Microbiology laboratories, and for the appropriate use of antibiotics.

In conclusion, urine culture positivity in the present study was 43.24%, of which Gram Negative Bacilli (GNB) constituted 86.93% and Gram Positive Cocci (GPC) constituted 12.96%. *Escherichia coli* was the commonest isolate in the present study- 44.71%, followed by *Klebsiella* species- 11.29%. Among the Gram Positive Cocci, Enterococcus species was the predominant isolate- 9.6% in the present study. ESBL production in the present study was found to be 42.79%. All the *Pseudomonas* isolates in the present study (n=16; 3.84%) were negative for ESBL production. ESBL production for the individual organisms in the present study was as follows- *E coli*- 45.16%, *Citrobacter* species- 23.8%, *Klebsiella* species- 17.02%, *Proteus* species- 14.28% and *Enterobacter* species- 12%. In conclusion the present study reinforces the need for the establishment of guidelines for early phenotypic detection of ESBLs in Microbiology laboratories, and for the appropriate use of antibiotics.

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References

1. L.S.Briongos-Figuero, T.Gomez-Traveso, P.Bachiller-Lugue, M.Dominguez-Gil Gonzalez, T.Palacios-Martin, A.Gomez Nieto et al. Epidemiology, Risk factors and Comorbidity for Urinary Tract Infections caused by Extended

Spectrum Beta Lactamase(ESBL)-producing Enterobacteria. International Journal of Clinical Practice, 2012; 66(9): 891-896.

2. Akram Hassan Mekki, Abdullahi Nur Hassan and Dya Eldin M Elsayed. Extended spectrum beta lactamases among multi drug resistant *Escherichia coli* and *Klebsiella* species causing urinary tract infections in Khartoum. Journal of Bacteriology Research, August 2010; 2(3): 18-21.
3. Dried Gram Negative Procedure Manual [package insert]. Camberly UK: Siemens Healthcare Diagnostics Ltd; 06/2013.
4. Johan Tham. Extended-Spectrum Beta-Lactamase- Producing Enterobacteriaceae: Epidemiology, Risk Factors, and Duration of Carriage. Climate Compensated Paper, 2012; 1-85.
5. Deepti Rawat and Deepthi Nair. Extended Spectrum Betalactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases, 2010 Sep-Dec; 2(3): 263–274.
6. Illamani V, Raveendran SR, Chitralekha S, Menezes CA. Evaluation of the association between the incidences of extended spectrum β-lactamase (ESBL) producing organisms in diabetic patients with recurrent urinary tract infections (UTIs). Journal of Pharmaceutical and Biomedical Sciences, 2013, January; 26(26): 278-282.
7. Neelam Taneja, Pooja Rao, Jitender Arora & Ashok Dogra. Occurrence of ESBL & Amp-C β-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res, January 2008: 85-88.
8. Supriya S. Tankhiwale, Suresh V. Jalgaonkar, Sarfraz Ahamad & Umesh Hassani. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian Journal of Medical Research, December 2004; 120: 553-556.
9. Dr IM Rejitha, Dr G Sucilathangam, Dr G Velvizhi. Urinary Tract Infection (UTI) In The Elderly - A Clinical and Microbiological Study. Indian Journal of Applied Research- Medical Science, April 2014; 4(4): 465-467.
10. Hasan Ejaz, Ikram-ul-Haq, Aizza Zafar, Saqib Mahmood and Muhammad Mohsin Javed. Urinary Tract Infections caused by Extended Spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*. African Journal of Biotechnology, 21st November 2011; 10(73): 16661-16666.
11. Meltem Isikgoz Tasbakan, Raika Durusoy, Husnu Pullukcu, Oguz Resat Sipahi, Sercan Ulusoy and 2011 Turkish Nosocomial Urinary Tract Infection Study Group. Hospital-acquired urinary tract infection point prevalence in Turkey: Differences in risk factors among patient groups. Annals of Clinical Microbiology and Antimicrobials, 2013; 12(31): 01-08.
12. Enas Sh. Khater and Hammouda W. Sherif. Rapid detection of Extended Spectrum Betalactamase (ESBL) producing strain of Escherichia coli in Urinary Tract Infections of patients in Benha University Hospital, Egypt. British Microbiology Research Journal, 2014; 4(4): 443-453.
13. Fereshteh Javadian, Zahra Sepehri, Hamideh Khaje, Raziyeh Farazmand, Zahra Miri, Naghmeh Gholipoura and Zahra Shahi. Detection, susceptibility and molecular characterisation of ESBL producing *E. coli* causing urinary tract infection. Journal of Biodiversity and Environmental Sciences , 2014; 5(1): 291-299.

14. L. F. Nimri and B. A. Azaizeh. First report of Multidrug resistant ESBL-producing urinary *Escherichia coli* in Jordan. British Microbiology Research Journal, 2012; 2(2): 71-81.
15. Babak Pourakbari, Farzad Ferdosian, Shima Mahmoudi, Mostafa Teymuri, Farah Sabouni, Hossein Heydari et al. Increase resistant rates and ESBL production between *E. Coli* isolates causing Urinary Tract Infection in young patients from Iran. Brazilian Journal of Microbiology, 2012: 766-769.
16. Shahanara Begum, Md Abdus Salam, Kh Faisal Alam, Nurjahan Begum, Pervez Hassan and Jalaluddin Ashraful Haq. Detection of Extended Spectrum β-lactamase in *Pseudomonas* species isolated from two tertiary care hospitals in Bangladesh. BMC Research Notes, 2013; 6(7): 1-4.
17. Dinesh Kumar, Amit Kumar Singh, Mohammed Rashid Ali and Yogesh Chander. Antimicrobial suscepbility profile of Extended Spectrum Betalactamase (ESBL) producing *Escherichia coli* from various clinical samples. Infectious Diseases: Research and Treatment, 2014; 7: 1-8.
18. Rohini P, Ambica R and Nagarathnamma T. The study of drug resistance and ESBL production in *Escherichia coli* causing urinary tract infections. International Journal of Current Research, September 2013; 5(9): 2602-2605.
19. Farhat Ullah, Salman Akbar Malik and Jawad Ahmed. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. African Journal of Biotechnology, 18 August, 2009; 8 (16): 3921-3926.
20. Rajan S, Prabavathy J. Antibiotic Sensitivity and Phenotypic Detection Of ESBL producing *E.Coli* Strains Causing Urinary Tract Infection In a Community Hospital, Chennai, Tamil Nadu, India. WebmedCentral PHARMACEUTICAL SCIENCES 2012;3(11): 1-15.
21. Datta P,Gupta V, Sidhu S, Chander J. Community Urinary Tract Infection due to ESBL producing *E coli:* Epidemiology and susceptibility to oral antimicrobials including Mecillinam. Nepal Journal of Medical Sciences, January-June 2014; 3 (1): 5-7.
22. Jafar Mobaleghi, Heiman Salimizand, Soheila Beiranvand, Shaho Membari, And Enayat Kalantar. Extended Spectrum β-lactamases in urinary isolates of *Escherichia coli* in five Iranian hospitals. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(2): 35-36.