Evaluation Of Charlson's Co-Morbidity Index And Risk Factors Among Patients With And Without Extended Spectrum B-Lactamase Producing Enterobacteriaceae Spp. Urinary Isolates In A Super-Specialty Hospital

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Abstracts: Background & Objectives: Extended-Spectrum Beta-Lactamase (ESBL) producing members of the family Enterobacteriaceae are emerging worldwide The aim of this study was to evaluate risk factors, co-morbidity status and short term mortality rates among hospitalized patients with and without ESBL producing Enterobacteriaceae spp. urinary isolates. Methods: An analytical cross-sectional study conducted in a super-specialty hospital from December 2014 to July 2015. Urine samples from 100 patients which repeatedly yielded significant colony counts of Enterobacteriaceae spp. isolates were identified using standard biochemical tests. Antibiotic susceptibility testing of these isolates was carried out by modified Kirby Bauer disk diffusion method as per CLSI guidelines 2014. Isolates which were resistant to cefotaxime and/or ceftazidime were tested for the production of ESBL by phenotypic confirmatory disc diffusion test. Relevant clinico-epidemiological details of these patients were subsequently obtained from Medical records as per the proforma formulated. The original version of the Charlson Index (CI) was used to assess co-morbidity and short term mortality rates. Results & Interpretation: Escherichia coli followed by Klebsiella pneumonia were the predominant isolates. 40 isolates were confirmed as ESBL producers. All isolates had Multiple Antibiotic Resistance (MAR) index of >0.2. The p-value of difference in proportion of all the risk factors distributed among patients with and without ESBL producing urinary Enterobacteriaceae spp. isolates respectively was found to be >0.05. The p-value of difference in mean Charlson index scores between these two groups of patients was 0.45. **Conclusions:** The results obtained in our study are largely inconclusive. It is imperative that more number of multicentre studies should be conducted in order to generate conclusive evidence on this subject. [Mohit B NJIRM 2016; 7(5):40-45]

Key Words: ESBL, Enterobacteriaceae spp., MAR index, Charlson's index

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Introduction: Extended-Spectrum Beta-Lactamase (ESBL) producing members of the family Enterobacteriaceae are emerging worldwide.[1-3] These dreaded organisms are associated with high morbidity and mortality rates in hospitalized patients and exhibit resistance to all penicillins, cephalosporins (including third and fourth generation agents) and monobactams like aztreonam. These organisms are also often cross-resistant to trimethoprim/sulfamethoxazole and fluoroquinolones. Risk factors for acquiring such infections include co-morbidity, frequent use of health resources, prior use of antibiotics, recurrent urinary tract infections (UTIs), older age and male sex.[2, 4]

There are limited number of studies which have assessed the specific risk of UTIs caused by ESBL-producing Enterobacteriaceae spp. This study was conducted with the aim of generating a hypothesis on probable association between isolation of ESBL producing members of the family Enterobacteriaceae from urine samples of hospitalized patients with co-morbidity status and subsequent short term mortality rate. A comparative evaluation of risk factors associated with isolation of both ESBL and non-ESBL producing Enterobacteriaceae spp. respectively, from urine samples of hospitalized patients was also attempted.

Methods: An analytical cross-sectional study was conducted in a super-specialty hospital from December 2014 to July 2015 after obtaining approval from institutional ethics committee. Urine samples received from 2439 consecutive hospitalized patients (admitted in wards and Intensive Care Units) were subjected to bacterial culture and sensitivity. Urine samples from one hundred patients which repeatedly yielded $\geq 10^4$ or $\geq 10^5$ CFU/ml (significant colony count in catheterized or self voiding patients respectively) of Escherichia coli, Klebsiella pneumoniae and Proteus spp. either singly or in various combinations to a maximum of two isolates per urine sample, were identified using standard biochemical tests.[5,6]

Antibiotic susceptibility testing of these isolates was carried out by modified Kirby Bauer disk diffusion method as per Clinical and Laboratory Standards

Institute (CLSI) guidelines 2014 using the following antibiotic discs: Amoxicillin-Clavulanic acid (20/10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), ciprofloxacin (30µg), ofloxacin (5µg), norfloxacin (10µg), nitrofurantoin (300µg), gentamicin (10µg), ceftriaxone (30µg), cefuroxime (30µg), cefoperazone (75µg), ceftazidime (30µg), cefotaxime (30µg), cefepime (30µg), piperacillin-tazobactam (100/10µg), imipenem (10µg) and meropenem (10µg). Multiple antibiotic resistance (MAR) index of all the isolates was calculated as per the procedure described by Krumperman by the following formula: Number of antibiotics to which the isolate was resistant/Total number of antibiotics against which the isolate was tested.[7] Isolates which were resistant to cefotaxime and/or ceftazidime were tested for the production of ESBL by phenotypic confirmatory disc diffusion test (as per CLSI guidelines 2014) using cefotaxime (30/10µg), cefotaxime-clavulanate (30/10 µg) discs and ceftazidime (30 µg), ceftazidime-clavulanate (30/10 µg) discs respectively. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate in comparison to the zone diameter of the agent when tested alone was considered as positive for ESBL production.

Relevant clinico-epidemiological details of these patients were subsequently obtained from Medical records as per the proforma formulated. Information regarding several risk factors like presence of urinary catheter, hospitalization in previous month, residence in an extended care facility, history of recurrent urinary tract infections (≥3 episodes per year), history of empirical administration of antibiotics, history of benign prostate hyperplasia (BPH) in male patients and results of blood culture (if done) was recorded. The original version of the Charlson Index (CI) was used to assess co-morbidity and short term mortality rate among patients with and without urinary ESBL producing Enterobacteriaceae spp. isolates respectively.[8]

Charlson Index consists of nineteen items corresponding to the following co-morbid conditions: Myocardial infarction,congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, COPD, connective tissue disease, peptic ulcer disease, diabetes mellitus, hemiplegia, leukemia, moderate to severe chronic kidney disease, malignant lymphoma,solid tumor, liver disease, Acquired Immuno Deficiency Syndrome (AIDS). Each of these conditions may increase mortality in patients based on their severity and is assigned a score. Age grouping of patients into five categories namely (<40, 41-50, 51-60, 61-70 and 71-80 years respectively) is done and each of these age groups is also assigned a score from 0 to 4 respectively. The sum of co-morbidity and age group scores (as applicable for different patients) is calculated. A score of 0–1 points signifies no co-morbidity, 2 points low and > 3 points high co-morbidity respectively. This score is also used to predict short term mortality rate of different patients as follows: 0 points: 12% mortality/year; 1–2 points: 26% mortality/year; 3–4 points: 52% mortality/year and > 5 points: 85% mortality/year respectively.[8]

Result: Fifty one out of the 100 urinary Enterobacteriaceae spp. isolates were obtained from male patients. The mean age (± S.D.) of the study population was 57.5±15.8 years. Out of 100 isolates, forty seven were Escherichia coli, forty three were Klebsiella pneumoniae and ten were Proteus spp. respectively. The antibiotic resistance profiles of these isolates has been depicted in Table 1. All isolates had MAR index of >0.2. The average MAR index of Escherichia coli, Klebsiella pneumoniae, Proteus spp. respectively has been depicted in Table 2. All 100 isolates were resistant to cefotaxime and/or ceftazidime. However, only forty of these isolates were confirmed as ESBL producers. Figures 1a & b depict the results of ESBL testing by phenotypic confirmatory disc diffusion method as per CLSI guidelines 2014. The percentage distribution of ESBL positive bacterial isolates has been shown in Figure 2. Table 3 shows the distribution of risk factors among patients with and without ESBL producing urinary Enterobacteriaceae spp. isolates respectively. The p-value of difference in proportion of all the risk factors distributed among patients belonging to either of these groups was calculated using Z-test & was found to be >0.05. Co-morbidity status and predicted short term mortality rate of patients with and without urinary ESBL producing Enterobacteriaceae spp. urinary isolates has been depicted in Table 4.

Table 1: Table depicting the antibiotic resistance profiles of all the urinary bacterial isolates

Antibiotics	Escherichia coli n (%)	Klebsiella pneumonia n (%)	Proteus spp n (%)
Augmentin*	35 (77.8)	30 (69.8)	9 (90)
Co-trimoxazole**	43 (92)	39 (93)	9 (90)
Ciprofloxacin	45 (95)	42 (98)	9 (90)

Ofloxacin	44 (94)	39 (93)	9 (90)
Norfloxacin	46 (97)	40 (95)	9 (90)
Nitrofurantoin	14 (30)	12 (28)	9 (90)
Gentamicin	46 (97)	42 (98)	9 (90)
Ceftriaxone	45 (95)	41 (96)	9 (90)
Cefuroxime	43 (92)	41 (96)	9 (90)
Cefoperazone	46 (97)	42 (98)	10 (100)
Ceftazidime	45 (95)	40 (95)	10 (100)
Cefotaxime	45 (95)	39 (93)	9 (90)
Cefepime	44 (94)	39 (93)	10 (100)
Piptaz***	32 (68.08)	29 (67.44)	10 (100)
Imipenem	39 (82)	41 (96)	9 (90)
Meropenem	46 (98)	42 (98)	9 (90)

*Amoxicillin-clavulanate; **Trimethoprim-sulfamethoxazole; ***Piperacillin-tazobactam

The mean Charlson index score (± S.D.) of patients with and without ESBL producing Enterobacteriaceae spp. urinary isolates was 2.82 ± 2.18 and 2.36 ± 1.64 respectively. The p-value of difference in mean Charlson index scores between these two groups of patients was also calculated using Z-test & was found to be 0.45.

Table:2 : Table depicting average MAR index of urinary bacterial isolates

Organisms	Average MAR Index
Escherichia coli	0.75
Klebsiella pneumonia	0.75
Proteus spp.	0.88

Table 5 shows the clinical features, blood culture results, outcome at the time of discharge from hospital and mean hospital stay among patients with and without urinary Enterobacteriaceae spp. ESBL producing urinary isolates respectively. Four out of 40 patients with ESBL producing Enterobacteriaceae spp. urinary isolates expired during their stay in the hospital. Bacterial isolates with similar antibiograms were repeatedly obtained from urine and blood samples of two of these patients. While blood and urine samples of one of these patients yielded Escherichia coli susceptible only to tigecycline and colistin, pan-drug resistant Klebsiella pneumoniae was isolated from both samples obtained from the other patient. Blood cultures of two other patients who expired were sterile. Three out of 60 patients without ESBL producing Enterobacteriaceae spp. urinary isolates also expired. However, blood cultures of all these patients were sterile.

Table 3: Table depicting the distribution of risk factors among patients with and without ESBL producing urinary Enterobacteriaceae spp. isolates

Risk factors	Patients with ESBL producing urinary Enterobacteriaceae spp. Isolates n (%)	Patients without ESBL producing urinary Enterobacteriaceae spp. Isolates n (%)	p value
Male gender	25 (62.5)	26 (43.3)	0.061
Age > 60 years	15 (37.5)	12 (40)	0.7872
Indwelling urinary catheter	36 (90)	50 (83.3)	0.3472
Hospitalization in previous month	10 (25)	14 (23.3)	0.8494
Residence in an extended care facility	2 (5)	1 (1.67)	0.3472
History of recurrent UTI	2 (5)	1 (1.67)	0.3472
History of empirical antibiotic administration	38 (95)	58 (96.7)	0.6744
History of BPH (male patients only)	2 (5)	1 (1.67)	0.3472

Table 4: Table showing the co-morbidity status and predicted short term mortality rate of patients with and without urinary ESBL producing Enterobacteriaceae spp. isolates

Co-morbidity status of 40 patients with ESBL producing urinary Enterobacteriaceae spp. isolates			Co-morbidity status of 60 patients without ESBL producing urinary Enterobacteriaceae spp. isolates		
No co-morbidity n(%) Score: 0-1	Low co-morbidity n(%) Score: 2	High co-morbidity n(%) Score: >3	No co-morbidity n(%) Score: 0-1	Low co-morbidity n(%) Score: 2	High co-morbidity n(%) Score: >3
5 (12.5)	28 (70)	7 (17.5)	10 (16.67)	42 (70)	8 (13.33)

Predicted short term mortality rate of 40 patients with ESBL producing urinary Enterobacteriaceae spp. isolates (%)				Predicted short term mortality rate of 60 patients without ESBL producing urinary Enterobacteriaceae spp. isolates (%)			
12%/year n(%)	26%/year n{%)	52%/year n(%)	85%/year n(%)	12%/year n(%)	26%/year n(%)	52%/year n(%)	85%/year n(%)
5 (12.5)	28 (70)	5 (12.5)	2 (5)	10 (16.7)	42 (70)	6 (10)	2 (3.3)

Table 5: Table showing the clinical features, blood culture results, outcome at the time of discharge from hospital and mean hospital stay among patients with and without urinary Enterobacteriaceae spp. ESBL producing isolates

Symptoms	Patients with ESBL producing urinary Enterobacteriaceae spp. isolates n (%)	Patients without ESBL producing urinary Enterobacteriaceae spp. isolates n (%)
Asymptomatic	10 (25)	15 (25)
Febrile syndrome	15 (37.5)	20 (33.3)
Micturition syndrome	15 (37.5)	20 (33.3)
Sepsis	20 (50)	10 (16.7)
[@]Others	25 (62.5)	15 (25)
Blood culture		
*Positive ESBL Enterobacteriaceae spp.	2 (5)	0 (0)
*Positive for any other micro organism	18 (45)	10 (16.7)
Negative	15 (37.5)	40 (66.7)
Not done	5 (12.5)	10 (16.7)
Outcome at discharge		
Alive	36 (90)	57 (95)
Dead	4 (10)	3 (5)
Mean hospital stay (± S.D.) in days	21.56 ± 12.34	21.49 ± 12.26

@Clinical features of involvement of other organ systems like brain, lungs, heart, gastro intestinal tract etc.
*Associated bacteraemia

Figures 1a & b: Figures showing ESBL negative and positive isolates respectively

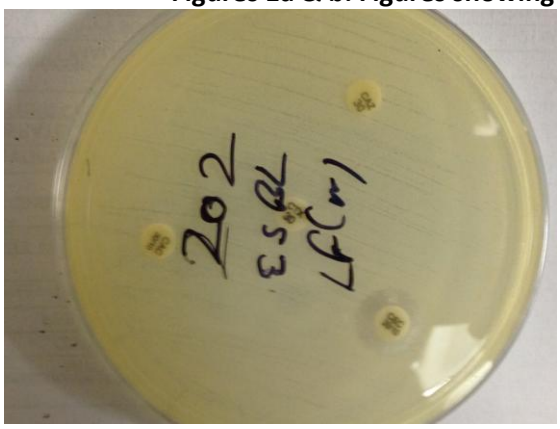
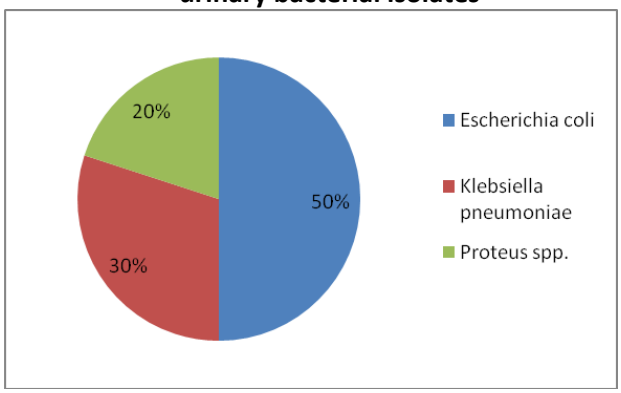


Figure 2: Percentage distribution of ESBL positive urinary bacterial isolates



Discussion: In the present study, majority of the urinary Enterobacteriaceae spp. isolates exhibited high degree of resistance to β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanate, piperacillin- tazobactam), fluoroquinolones (ciprofloxacin, ofloxacin and norfloxacin), cephalosporins (cefotaxime, ceftazidime, cefuroxime, ceftriaxone, cefoperazone, ceftriaxone, cefepime), trimethoprim-sulfamethoxazole, aminoglycosides (gentamicin). However, barring Proteus spp., Escherichia coli and Klebsiella pneumoniae were relatively sensitive to nitrofurantoin. Nitrofurantoin is active against most common uropathogens, but most Proteus species, Serratia marcescens, and Pseudomonas aeruginosa are naturally resistant.[9,10] ESBLs are usually ineffective against cephamycins (eg. cefoxitin and cefotetan) and carbapenems (eg. imipenem, meropenem and etrapenem).[11] These enzymes are sensitive to β-lactamase inhibitors (sulbactam, clavulanic acid, and tazobactam).[12] However, in our study the degree of resistance to carbapenems and β-lactam/β-lactamase inhibitor combinations was high for all the isolates irrespective of their ESBL production status. This could probably be due to the co-existence of several other antibiotic resistance mechanisms, which were not tested for in the present study.

The average MAR index of Escherichia coli, Klebsiella pneumoniae and Proteus spp. respectively was >0.2 each. MAR index values >0.2 indicate high risk source of contamination where antibiotics are often used.[7] It is a well known fact that inadvertent use of antibiotics is often associated with emergence of multi-drug resistant strains of bacteria both in the community and hospital settings. This fact has also been highlighted in this study as all subjects in the study population had received antibiotics empirically.

There was no significant difference in the risk factors among patients with and without ESBL producing urinary Enterobacteriaceae spp. isolates. Also, the difference in mean Charlson index scores between these two groups of patients was not found to be statistically significant. In a study conducted by Briongos-Figuero L.S. et al, these results were however found to be statistically significant.[2] This difference in results could be attributed to the difference in study designs (while ours was a cross-sectional study, the latter was a case-control study) and small sample size. The results of analytical cross-sectional studies need to be interpreted with caution, because both outcome and exposure are measured simultaneously and it may not be possible to know which preceded the other.[13]

Conclusion: As the number of studies exploring the possibility of an association between ESBL production and associated co-morbidity and mortality are very few, it is imperative that the results obtained in all these studies including ours should not be considered as final. More number of multicentre case-control studies should be conducted in order to generate conclusive evidence on this subject.

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