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PREVALENCE OF DRUG RESISTANCE IN ESBL PRODUCING ESCHERICHIA COLI CAUSING UTI IN RURAL TERTIARY CARE HOSPITAL FROM HARYANA, INDIA

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ABSTRACT

Background: Antibiotic resistance is a problem of deep scientific concern in members of *enterobacteriaceae*. Moreover, the emergence of antibiotic resistance is inevitable and thus, is considered as a major public health problem in the treatment of bacterial infections both in hospital as well as community settings. **Aims:** The widespread presence of antibiotic resistance among *e. Coli* in our environment necessitates the rapid detection and monitoring of the antibiotics susceptibility trends in the clinical isolates for the judicious management and effectively delineating guidelines to maintain the desired effectiveness of antibiotics. In addition to that, the production of extended-spectrum β -lactamases (esbls) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by enterobacteriaceae and is a potential threat to the currently available antibiotic armory. Thus, the present study was aimed to know the prevalence and antimicrobial resistance among esbl producing *e. Coli* from urine samples. **Materials and Methods:** Urine samples were obtained from both inpatient and outpatient with complaint of uti at our rural tertiary care referral centre. All the urine samples were analyzed for isolation and identification of *e. Coli*. Antimicrobial susceptibility testing of *e. Coli* isolates were validated following the kirby-bauer disc diffusion technique. Screening test for esbl was done as per the recommendation of the nccls. **Results:** A total of 99 isolates of *e. Coli* from urine samples were obtained. Of these 36 (36.03%) were esbl producers and 63 were non-esbl producers. The esbl producing strains were more resistant than non-esbl producing strains. Among esbl producers the resistance pattern for 3gcs was highest for ceftriaxone (97.22%) followed by cefotaxime (88.88%) and ceftazidime (83.33%). The esbl producing isolates were significantly resistant ($p < 0.05$) to amikacin (16.66%), ciprofloxacin (83.33%) and norfloxacin (94.44%) as compared to non-esbl producers. More effective drugs against esbl producers were imipenem with 100% sensitivity followed by amikacin (83.34%) and nitrofurantoin (80.55%). **Conclusion:** In conclusion, our study strongly reinforces the necessity for appropriate use of antibacterial compounds, especially in case of esbl producers. In the present study, the most effective drug among esbl producers and non-esbl producers was imipenem. Furthermore, in the present context, it should be done at the genetic level, to monitor more detailed patterns of emergence for the welfare of patients.

Keywords: *E. coli*; UTI; AST; ESBL; 3GCss

INTRODUCTION

Escherichia coli are one of the common etiological agents of Urinary tract infections (UTIs), with an estimated burden of more than 250 million cases annually. Thus, remains an alarming problem for both patients as well as health care agencies (Ronald *et al.*, 2001).

Among suspected UTI cases, antibiotic treatment is most often initiated empirically, even before the urine culture results are available. Unfortunately, antibiotic resistance has become an increasingly critical problem even in developing countries like India (Sumeeta *et al.*, 2002; Tankhiwale *et al.*, 2004). Furthermore, it has been observed that antibiotic susceptibility pattern of bacterial isolates does not remain constant, but dynamics might vary with time and environment (Hassan *et al.*, 1985). Most often the therapeutic steps of UTIs treatment, involves a short term course of antimicrobial drug, such as antibiotics viz. ampicillin, chloramphenicol, colistin methane sulphonate, kanamycin, nalidixic acid, nitrofurantoin, streptomycin, norfloxacin, trimethoprim-sulfamethoxazole etc. Moreover, a significant

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emerging problem is the extended spectrum beta-lactamase (ESBL) mechanism of resistance (Andrade *et al.*, 2006). ESBLs are beta-lactamases that hydrolyze extended spectrum cephalosporins with an oxyimino side chain (Akram *et al.*, 2007). ESBL producers are now one of the main gram-negative species to cause infections with ESBL producing bacteria in humans (Astal *et al.*, 2004; Pitot *et al.*, 2005). The increase of drug resistance among these organisms has made UTI treatment difficult and has led to greater use of expensive broad spectrum antibiotics such as third generation cephalosporins. Therefore, systematic monitoring of such resistance at local, national and international levels is recognized as an integral part of the control strategy by most national and international organizations (O'Brien, 1997). Thus, there is an urge for an efficient surveillance system to monitor the possible current occurrence and phenotypes of ESBL producing *E. coli* among UTI isolates. Therefore, the aim of the present study was to know the prevalence as well as antimicrobial resistance pattern among ESBL producing isolates of *E. coli* from urine samples.

MATERIALS AND METHODS

Study Design: Prospective study was designed to know the prevalence as well as antimicrobial resistance pattern among ESBL producing *E. coli* isolates from urine samples.

Study Subjects & Samples: The present study was conducted on UTI suspected cases of all age groups, of both sexes attended or admitted to various OPDs or wards of the BPS GMC for W, Khanpur Kalan, Sonapat, Haryana. One thousand seven hundred sixty urine samples were obtained consecutively from patients enrolled under study. Only one isolate per patient was included in the study.

Study site: BPS. G.M.C. Sonapat, Haryana.

Duration of study: Study was conducted over a period of 3 months (Jan 2014 to March 2014).

Inclusion criteria: Patients of all groups with clinical suspicion of UTI.

Exclusion criteria: Urine culture findings other than *E.coli* or growth of more than one type of microorganism were excluded from the study.

Methodology: All the urine samples were cultured on blood agar (Hi-Media, Mumbai, India) and MacConkey agar (Hi-Media, Mumbai, India) and incubated at 37°C for 16–18 hours. Isolates were identified on the basis of colony morphology and biochemical reactions (Mackie and Mc Cartney, 2007). All the clinical isolates (*E. coli*) were subjected to AST following modified Kirby Bauer disc diffusion method with third generation cephalosporins (3GCs) (NCCLS, 2011). The 3GCs included in the study were ceftazidime, cefotaxime and ceftriaxone each 30µg. Followed by, the incubation for 16–18 hours at 37°C. Isolates found resistant or with decreased susceptibility to any one of these 3GCs were selected for the presence of ESBLs. For each of the isolate antibiotic susceptibility testing was determined for different antibiotics: Amikacin (30µg), Cotrimoxazole (1.25/23.75µg), Ciprofloxacin (25µg), Gentamicin (10µg), Imipenem (10µg), Levofloxacin (10µg), Nitrofurantoin (30µg) and Norfloxacin (10µg).

Testing for presence of ESBL: ESBL detection was carried out in two steps first is Screening for ESBL producers by Double disc synergy test (DSST) followed by Phenotypic confirmatory test as per the recommendation of CLSI (CLSI, 2012). *E. coli* ATCC 25922 non-ESBL producing strain was used as a positive control.

Statistical analysis: The qualitative variables were compared using the Chi-square test (χ^2) of the SPSS software, version 20 and p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Emergence and re-emergence of antimicrobial resistance shows the way to therapeutic failure especially of empirical therapy. The problem is further substantially enhanced throughout the world with the emergence of ESBL strains. For that reason, the information about the prevalence of local and surrounding pathogens and their antimicrobial susceptibility pattern regarding their sensitivity and resistance are vital for clinicians' to treat patients effectively.

The present study demonstrates the antibiotic susceptibility pattern and ESBL prevalence among *E. coli* isolated from 1760 urine samples obtained consecutively (non-duplicate) from various indoor and outdoor

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patients. Out of these only 330 resulted to be culture positive that included 258 and 73 gram positive and negative respectively. Ninety nine of these 258 gram negatives were *E. coli* that further included in the study. Of these 99 *E. coli* isolates, 38 were male and 61 were females (Table 1).

Table 1: Gender wise distribution among ESBL and Non-ESBL producing *E. coli*

Gender	ESBL producers	non-ESBL producers	Total	p-value
Male	17(47.22%)	21 (33.33%)	38 (38.38%)	0.172
Female	19 (52.77%)	42 (66.66%)	61 (61.61%)	
Total	36	63	99	

Forty one of these showed resistance or decreased susceptibility to any one of the 3GCs. Among these the resistance pattern for 3GCs was highest for ceftriaxone (97.22%) followed by cefotaxime (88.88%) and ceftazidime (83.33%). Thirty six of these 41 were confirmed ESBL producers by double disc synergy test and phenotypic confirmatory test. In the present study, the presence of ESBL producers among clinical isolates was 36.36%, which is near to that of Tankhiwale *et al.*, (2004) from Wardha, India also reported a high proportion of ESBL producers from their study that included 41.3% were *E. coli* and 44.7% *Klebsiella pneumoniae*. Chatterjee *et al.*, (2012), from eastern India reported (31.6%) ESBL producers *E.coli* strains from inpatients. Falagas *et al.*, (2014) in a systematic review reported that in African countries the proportion of ESBL-producing isolates was > 15% in 10 out of 26 studies. However, the presence of ESBL producers among *E.coli* isolates from developing countries are less than 5% as reported by Sharifian *et al.*, (2006); Randrianirina *et al.*, (2007); and NCCLS., (2011).

However, from north India, Mathur *et al.*, (2002) reported ESBL rates as high as 58%. They have explained that the probable reason for the high rate of ESBL producers was a long term hospital stay that resulted in increased susceptibility to nosocomial infections and drug induced pressure. Pourakbari and coworkers (2012) reported that 37% of ESBL producing isolates included 42% of nosocomial and 32% isolates from community in Iran. Similarly, in the present study, 55.55% of the ESBL producers were from indoor patients and were under pressure of different antibiotics, leading particular drug resistance. Thus, it is worth it to state that throughout the world the prevalence of ESBL producers may vary profoundly and patterns change rapidly over time.

Moreover, our findings of maximum ESBL detection rate by ceftriaxone followed by cefotaxime and lastly ceftazidime showed acquiescence with that of Datta *et al.*, (2004). However, some researchers reported ceftazidime as the most effective in detection of ESBL producers (Tsering *et al.*, 2009).

There was no association between gender and ESBL producers and non-ESBL producers in the present study (Table 1). Furthermore, our finding suggested more number of females compared to males as ESBL producers were in agreement with that of Manjunath *et al.*, (2011); Kalantar *et al.*, (2012) and Sasirekha *et al.*, (2013) but not in concordance with that of Bajpai *et al.*, (2014).

Out of thirty six ESBL producing *E. coli*, 20 (55.55%) were from indoor patients and 16 (44.44%) were from outdoor patients [Table 2 (a) & (b)]. The percentages of ESBL producers among inpatients were higher in comparison to that of outpatients and the finding was in accordance with that of Singh *et al.*, (2013). Moreover, the highest numbers of ESBL producing isolates were from gynaecological and surgical departments (indoor & outdoor both). This finding is in agreement with that of Kateregga *et al.*, (2015).

Table 2(a): Distribution of ESBL producers among different Inpatients (Indoor) departments

S. No.	Departments (Inpatients)	No. of Isolates (Indoor) (n = 20)
1	Surgery ward	4
2	Gen. Med. ward	5
3	Orthopedics ward	2
4	Obs. & Gyne ward	9

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Table 2(b): Distribution of ESBL producers among different Outpatients (Outdoor patient)

S. No.	Departments (Outpatients)	No. of Isolates (Outdoor) (n = 16)
1	Syrgery	3
2	Obs. & Gynecolgy	4
3	Medicine	4
4	Orthopedics	3
5	Pediatrics	2

The population under study is broadly divided into 4 age groups. Fifteen of these 36 isolates were between the age group of 21 – 40 years of age. This age group included 13 females and 02 male, i.e. majority of the females were from the reproductive age group. Among the male ESBL producers 10 out of 17 were from the age group of 61 – 80 (Table 3). There was no patient in age group > 80 years. In addition, statistically significant numbers of females were there below 41 years of age; the probable reason for this is that 11 of the 13 females were from Obs. & Gyne. department. However, numbers of males were prominent above 41 years of age. Among them, 10 (> 61 years) of the 12 had already undergone one or the other kind of surgery and were under follow-ups.

Table 3: Age wise distribution of Male and Female among ESBL producers

Age group	Male	Female	Total
1 – 20	3	0	03
21 – 40	2	13	15
41 – 60	2	5	07
61 – 80	10	1	11

Furthermore, the ESBL producers were found to be significantly more resistant to antimicrobial agents, including amikacin (16.66%), ciprofloxacin (83.33%) and norfloxacin (94.44%). In addition, the resistance pattern among ESBL producers was norfloxacin (94.44%), ciprofloxacin (83.33%), cotrimoxazole (77.77%), gentamycin (58.33%), levofloxacin (55.55%), nitrofurantoin (19.44%) and amikacin (16.66%). Imipenem (100%) was found to be the most effective antibiotic against ESBL producers followed by amikacin, nitrofurantoin, levofloxacin, gnetamycin, cotrimoxazole, ciprofloxacin and norfloxacin (Table 4).

Table 4: Antibiotic resistant patterns of ESBL and non-ESBL producers

S. No	Drugs	ESBL Resistant (n=36)	Non-ESBL Resistant (n = 63)	p-value
1	Amikacin	06 (16.66%)	02 (3.17%)	0.018
2	Cotrimoxazole	28 (77.77%)	37 (58.73%)	0.055
3	Ciprofloxacin	30 (83.33%)	22 (34.92%)	0.000
4	Gentamicin	21 (58.33)	24 (38.09)	0.052
5	Imipenem	00	00	N/A
6	Levofloxacin	20 (55.55%)	26 (41.26%)	0.170
7	Nitrofurantoin	7 (19.44%)	6 (16.66%)	0.160
8	Norfloxacin	34 (94.44%)	41 (65.07%)	0.001

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On the other hand, among non-ESBL producers there was less resistance to the antibiotics and the highest resistance was against norfloxacin (65.07%) which was significantly ($p < 0.05$) lower than ESBL producing isolates. This was followed by resistance to cotrimoxazole (58.73%), levofloxacin (41.26%), gentamycin (38.09%), ciprofloxacin (34.92%), nitrofurantoin (16.66%) and amikacin (3.17%). The resistance to amikacin and ciprofloxacin were found to be significantly ($p < 0.05$) lower than that of ESBL producer and the remaining drugs were not significantly ($p > 0.05$) associated. Among non-ESBL producers also the most sensitive drug was imipenem (100%) followed by amikacin and nitrofurantoin respectively (Table 4).

Thus, in the present study, ESBL producing isolates were significantly more resistant to amikacin ($p < 0.01$), ciprofloxacin ($p < 0.01$) and norfloxacin ($p < 0.01$) as compared to non-ESBL producing isolates this finding is in accordance with other researchers (Oteo *et al.*, 2002; Tolun *et al.*, 2004). Moreover, association between ESBL producers and decreased susceptibility to fluoroquinolones (norfloxacin, ciprofloxacin) is in agreement with the findings of Spanu *et al.*, (2002). However, carbapenems (imipenem) showed the highest susceptibility patterns against both ESBL producers and non-producers isolated and this finding is in accordance with that of Akram *et al.*, (2007). Furthermore, the significantly higher sensitivity for amikacin was also in concordance with that of Bamford *et al.*, (2012).

One of the imperative findings of the present study was that, there was a coexistence of resistance among 3GCs and other drugs; this was also in concordance with that of Duttaroy *et al.*, (2005) and Tsering *et al.*, (2009) thus, indicating the multidrug resistance among isolates. However, the mechanism behind the coexistence of resistance is still ambiguous. The possible explanation for the association between ESBL production and fluoroquinolones (ciprofloxacin, norfloxacin) resistance (MDR) is the presence of genes of the two resistance mechanisms on the same plasmid. Besides this, the association between resistance to third-generation cephalosporins and fluoroquinolones, including active efflux and outer membrane protein alterations are the other potential explanations (Hoper., 2001; Deguchi *et al.*, 1997 and Livermoore and Paterson., 2006). In the present study, MDR was 56% and 34% among ESBL producers and non-ESBL producers. Likewise, Tsering *et al.*, (2009) reported that among ESBL and non-ESBL producers MDR was 69.14% and 21.66% respectively. Daoud *et al.*, (2003) and Jones *et al.*, (2003) also reported that ESBL producers are more prone to show MDR. Furthermore, so far ESBL has already been reported in hospitals throughout the world, thus it is indispensable to know the prevalence of ESBL producers of a particular hospital or community, so that a guideline can be formulated for empirical therapy. In addition, knowledge of the sensitivity and resistance patterns drugs for particular strains in a specified hospital or community help to channelise appropriate and judicious antibiotic use.

Conclusion

To conclude, the prevalence of ESBL in *E. coli* was found to be 36.05% in our tertiary care hospital setting which cannot be overlooked. Imipenem followed by amikacin remains the drug of choice for both ESBL and non-ESBL producers with the highest sensitivity. In addition, to that the ESBL producing isolates were more resistant than non-ESBL producers which is again an alarming problem need to be monitored in routine at the earliest. Furthermore, the major limitation of the present study was that we could not use any advanced molecular methods because of infrastructure related constraints.

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Declaration of Interest

The authors report no conflicts of interest.

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