Original Article

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Microbiology Section

Extra-intestinal β-lactamase Producing *Escherichia coli* Infection-an Emerging Infection in a South Indian Tertiary Care Hospital

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ABSTRACT

Introduction: In the recent years, extra-intestinal *Escherichia coli* infection has been a very important cause of mortality and morbidity. However, only a limited number of literatures are available on the clinical presentation and the outcome of the extra-intestinal pathogenic *E.coli* (ExPEC) infections. We investigated the prevalence, risk factors, anti-biogram and the outcome of the antibiotic treatment of the extra-intestinal infections caused by *E.coli* among hospitalized patients. This descriptive study was carried out in a multispeciality, tertiary care hospital.

Methods and Material: Two hundred ExPEC infected patients were included in the study. The demographic data, risk factors, details of the organ failure, anti-biogram, treatment and the outcome were collected in a structured pro forma. The severity was assessed by the APACHE II Score. The *E.coli* isolates were microbiologically characterized as Extended Spectrum β lactamase (ESBL) producers if they were found to be resistant to penicillin and the cephalosporins.

Statistical analysis: The proportions were expressed as percentages. The categorical data between the infection with the ESBL producers and the non-ESBL producers were compared by using the Chi-square test. The statistical analysis was performed by using SPSS, version 17.0

Results: Out of the 200 *E.coli* isolates, 132(66%) were extended spectrum beta lactamase producers. Diabetes mellitus (DM) was the most important risk factor for the ExPEC infection. In the anti-biogram, a high degree of resistance was seen against ampicillin (84%), the fluoroquinolones (71%), the 3rd generation cephalosporins (66%), the sulfonamides (58.5%), and the aminoglycosides (41%). Carbapenam resistance was seen in 8% of the isolates. For the treatment, the most widely prescribed antibiotics were the β-lactam+β-lactamase inhibitor combinations (39%) and the 3rd generation cephalosporins (18.5%). 65.15% patients improved with proper treatment, 15.9% patients expired (p=0.02) and 16.5% patients relapsed. There was no correlation between the risk factors, ages of the patients, the APACHE II score, organ failure and the ESBL producers. However, an increased mortality was seen in patients with blood stream infections and lung infections which were caused by *E. coli*.

Conclusions: The ExPEC Infection is associated with a high level of drug resistance, mortality, morbidity and relapse. The early use of the appropriate empirical antibiotics will probably reduce the mortality and the morbidity in these patients. The 8% carbapenam resistance implies that the organisms which produce carbapenemase (superbug) also infect our patients and this may emerge as a major cause of morbidity and mortality among the patients with ExPEC in the future.

Key Words: ExPEC, Host factors, Drug resistance, ESBL, Outcome

INTRODUCTION

In this era in which the health news often sensationalizes uncommon infection syndromes or pathogens, the strains of *Escherichia coli* that cause extra-intestinal infections are an increasingly important endemic problem and they are underappreciated as "killers" [1]. Extra-intestinal pathogenic *Escherichia coli* (ExPEC) are responsible for urinary tract, intra-abdominal and soft tissue infections, meningitis, pneumonia and osteomyelitis and they are often associated with bacteraemia [2,3]. The treatment of the *E.coli* infections is increasingly becoming difficult because of the multi-drug resistance exhibited by the organism. The extended spectrum β lactamase (ESBL) producing organisms pose a major problem in the clinical therapeutics [4]. Although extensive laboratory data supported by molecular methods are available on the virulence and resistance of ExPEC, clinical data, especially from developing countries are not available [5-9]. The knowledge of the clinical details, the treatment and the drug resistance pattern in a geographical area will help in the formation of an appropriate hospital antibiotic policy which can assist the clinicians in controlling these infections. Hence, we conducted this study to know the frequency of the extended Spectrum-β-lactamase producing *E.coli* and to co-relate the clinical details, treatment and the outcome with the drug resistance pattern of the extra-intestinal *E.coli* infections.

MATERIAL AND METHODS

This study was carried out in a multispeciality, tertiary care hospital at Mangalore. A total of 200 patients of all age groups, who were hospitalized with the ExPEC infection during the period from May 2010 to July 2011, who satisfied the following inclusion/exclusion criteria, were included in the study.

Inclusion criteria: Those subjects extra-intestinal clinical samples grew *E.coli* were included in the study.

Exclusion criteria: Subjects who received anti-microbial drugs during the past one month, those who had asymptomatic UTIs and polymicrobial infections were excluded from the study.

Sample size Calculation: By assuming that 65% of the hospital isolates were ESBL producers, a sample size of 200 was calculated by using the formula, $n = Z\alpha^2 pq / L^2$ (power is 90%).

METHODS

After obtaining the institutional ethics committee's clearance, the clinical data from the patients records were collected in a structured proforma. The data included the demographic details, risk factors (Diabetes mellitus, malignancy, post-surgical, on steroids), signs and symptoms, organ failure, details of the antibiotics which were used and the clinical outcome. The severity was assessed by the APACHE II Scores.

The patients whose samples like urine, blood, wound swab, pus, CSF, ascitic fluid or intravascular devices grew *E. coli*, were selected for the study.

Isolation and Identification of the Pathogens

The samples were processed immediately by using standard procedures. The isolates were identified, based on their colony morphologies on blood agar, Mac Conkey's agar and gram staining and by standard biochemical tests [5]. The blood isolates were identified by using the biochemical system, Vitek 2 (bioMerieux).

Anti-microbial Susceptibility Testing

The anti-microbial agents were tested by using the modified Kirby-Bauer disk diffusion method, in accordance with the CLSI guidelines [10]. The antibiotic disks (Hi Media, Mumbai) which used were ampicillin, piperacillin, Piperacillin + tazobactam, ceftriaxone, cefotaxime, ciprofloxacine, norfloxacin, amikacin, gentamicin, co-trimoxazole, Cefoperazone+sulbactam, imepenem and meropenem.

Screening for the ESBL Production

The isolates which were resistant to one or more of the third generation cephalosporins were tested for ESBL production by combination disk method using cefotaxime (30 µg), cefotaxime/clavulanic acid (10µg) ceftazidime (30µg) and ceftazidime/clavulanic acid (10µg). A ≥5mm increase in the diameter of the inhibition zone of the cephalosporin-plus-clavulanate disc as compared to that of the cephalosporin disc alone, was interpreted as a phenotypic evidence of the ESBL production [10]. A carbapenem resistance was noted.

Statistical Analysis

The data was expressed as percentage or mean +S.D. The Chi-square test was used to find the association between the drug resistance (ESBL production) and the demographic details, risk factors and the outcome. A p value of <0.05 was considered as significant. The analysis was performed by using the statistical package, SPSS, version 17.0 (SPSS,USA).

RESULTS

In total, 200 patients with extra-intestinal *E.coli* infections were selected. Out of these, 103 (51.5%) had UTIs, 50 (25%) had bacteraemia, 29(14.5%) had wound infections, 13(6.5%) had pneumonia, 3(1.5%) had intra-vascular device infection and 2 (1%) had meningitis. Out of the 200 *E.coli* isolates, 132(66%) were ESBL producers [Table/Fig-1]. A majority were community acquired infections [176 (88%)] and 24 (12%) were hospital acquired infections. There was no difference in the demographic data and the risk factors of the patients who were infected with

Descriptive data	ESBL producing isolates	Non ESBL producing isolates
Demographic details:	(N=132)	(N=68)
Gender:		
Male: (N=115)	82(62.1%)	33(48.5%)
Female: (N=85)	50(37.9%)	35(51.5%)
Age:		
< 18 (N=7)	4(3.03%)	3(4.4%)
18-44 (N=46)	26(19.69%)	20(29.4%)
45-59 (N=55)	41(31.06%)	14(20.6%)
>60 (N=92)	61(46.2%)	31(45.6%)
Type of EXPEC infection:		
UTI: (N=103)	63(47.7%)	40(58.8%)
Sepsis: (N=50)	33(25%)	17(25%)
Wound: (N=29)	21(15.9%)	8(11.8%)
Pneumonia: (N=13)	11(8.3%)	2(2.9%)
Meningitis (N=2)	2(1.5%)	0%
Intra vascular device (N=3)	2 (1.5%)	1(1.50%)
APACHE II score: (mean)	±9.80	±7.71
Risk factors:		
Diabetes mellitus: (N= 79)	56(42.4%)	23(33.8%)
Malignancy: (N=25)	19 (14.4%)	6(8.8%)
Post surgical: (N=32)	20(15.15%)	12(17.6%)
On steroids: (N=23)	12(9.1%)	11(16.2%)
Complication:		
Renal failure: (N=57)	42(31.8%)	15(22.10%)
Liver failure: (N= 32)	23 (17.40%)	9(13.30%)
Hypotension: (N= 8)	5 (3.78%)	3 (4.34%)
ARDS: (N= 5)	4 (3.03%)	1(1.44%)
Treatment:		
β lactamase inhibitors: (N=78)	67(51%)	11(16%)
Cephalosporin: (N=46)	23(17.4%)	23(34%)
Carbapenems: (N= 31)	21(15.9%)	10(15%)
Aminoglycoside: (N=14)	10 (7.6%)	4(6%)
fluoroquinolones: (N=17)	7(5.3%)	10(15%)
Others (N=8)	0%	8(12%)
Trimethoprim/ sulfamethoxazole (N=6)	4(3%)	2(2.94%)
Outcome:		
Improved: (N=138)	86(65.15%)	52(76.5%)
Relapses: (N=33)	20(15.15%)	13(19.1%)
Expired: (N=22)	21(15.90%)	1(1.5%)
Lost to follow up: (N=7)	5(3.8%)	2(2.9%)
ICU admission: (N=40)	35(87.5%)	5(12.5%)

[Table/Fig-1]: Patient characteristics with ESBL and non-ESBL producing *E.coli* isolates

the ESBL producing *E.coli* vs the non-ESBL producing *E.coli* [Table/Fig-1].

Out of the 200 patients, 57 (28.5%) had renal failure, 32(16%) had hepatic failure, 72 (36%) had a low haemoglobin value, in 15 (7.5%) patients, the peripheral smear reports showed an evidence of intravascular haemolysis and 5(2.5%) patients developed ARDS after infection with ExPEC [Table/Fig-1]. Some of the patients had no complications.

Analysis of drug resistance pattern among the 200 *E.coli* showed that a majority (84%) were resistant to ampicillin, followed by ciprofloxacin (71%), ceftriaxone (65%), co-trimoxazole (58%) amikacin (41%) and piperacillin+tazobactam (28.3%) and only a minority were resistant to cefoperazone+sulbactam (15%) and imepenem (8%). The ESBL producing isolates also showed a high degree of resistance to the other non β lactam classes of antibiotics [Table/Fig- 2].

The antibiotics which were prescribed were β lactam + β lactamase inhibitors [78 (39%)], Cefoperazone+sulbactum [52(26%)], Piperacillin + tazobactam [17(8.5%)], cephalosporins [46 (23%)], Carbapenems [31(15.5%)], fluoroquinolones [17(8.5%)], Aminoglycosides [14(7%)], Trimethoprim/sulfamethoxazole [6(3%)] and others (nitrofurantoin and chloramphenicol) [8(4%)] [Table/Fig-3]. Among patients who were infected with ESBL producing isolates, the most widely used antibiotics were the β lactam+β lactamase inhibitor combinations (51%)[Table/Fig-1]. For the carbapenemase producing isolates, the most widely used antibiotics which were used were cefoperazone +sulbactam and piperacillin + tazobactam.

OUTCOME

In our study population, maximum mortality was observed in the bacteraemic patients, followed by those with pneumonia, intravascular device infection and meningitis [Table/Fig-4]. Mortality was mainly associated with ESBL producing isolates [Table/Fig-5].

DISCUSSION

The results of our study have shown that out of the 200 hospitalized patients with the ExPEC infection, 132(66%) were ESBL producers. Other studies from India have reported a 50%-70% prevalence of the ESBL production among the *E.coli* [5,7-9], However, the clinical details and the outcome of the ESBL producing *E.coli* infected patients were not studied.

The anti-microbial resistance in *E.coli* has significant implications in the empirical therapy, because it is associated with worse outcomes for the patients with bacteraemia [11].

A majority of our patients were elderly patients and a male preponderance was seen. Several studies have shown that females were more vulnerable to these infections [12,13].

We found diabetes and malignancy as the important risk factors, in addition to immunosuppression/the immunocompromised state in our study. Several other studies also reported that DM and malignancy were the two important risk factors for the ExPEC infections [12-15]. We also found a higher prevalence of the ESBL producing isolates in the DM patients, which was nearly 4 times as compared to that in non diabetics. In malignant patients also, the risk was 4 fold. This may be because of the frequent antibiotic use due to the recurrent infections.

	ESBL Producers	Non ESBL Producers
Fluoroquinolones	109 (82.5%)	19 (28%)
Sulfonamide	77 (58.5%)	15 (22%)
Aminoglycosides	73 (55%)	5 (7%)

[Table/Fig-2]: Resistance pattern of ESBL Vs non ESBL producing *E.coli* isolates to various non β- lactam antibiotics classes :

	numbers
Carbapenems:	31
Cephalosporins:	
4th generation:	4
3rd generation:	37
2nd generation:	3
1st generation:	2
B-lactam+ β lactamase inhibitors:	
Cefoperazone+sulbactum:	52
Piperacillin+ tazobactam:	17
Amoxicillin +clavulanic acid:	6
Ceftriaxone+ tazobactam:	3
Aminoglycosides:	
Amikacin:	10
Gentamicin:	4
Trimethoprim +sulfamethoxazole:	6
Fluoroquinolones:	17
Nitrofurantoin:	5
Chloramphenicol:	3

[Table/Fig-3]: Prescribed antibiotics: (N=200)

Infection	Improved	Relapses	Expired	Lost to follow up
UTI (N=103)	72	27	2	2
Sepsis (N= 50)	30	4	13	3
Wound (N=29)	22	4	2	1
Pneumonia (N=13)	9	1	3	0
Meningitis (N=2)	1	0	1	0
Others (intra vascular Device) (N=3)	0	0	2	1

[Table/Fig-4]: Outcome of EXPEC infection (N=200)

Drug resistance	Expired	Survived
ESBL producer	21	111
Non ESBL producers	1	67
Carbapenemase producers	2	14

[Table/Fig-5]: Outcome of ExPEC infection with ESBL and non ESBL producer
ESBL: P=0.002; Carbapenemase: p=0 .842.

Although there was no difference in the APACHE II scores in the patients who were infected with the ESBL producers and the non ESBL producers, a majority of the patients who were infected with the ESBL producing *E.coli*, had organ failure vs the patients who were infected with the non ESBL producing *E.coli* (renal failure: 74 vs 26%; hepatic failure: 72 vs 28%), thus indicating that sepsis and multi-organ failure were common, which led to more severe infections. This may be because of the delay in the use of the appropriate antibiotics, which might have led to the sepsis.

Previous studies have shown that the ESBL producing organisms are also frequently resistant to the non β-lactam antibiotics such as the fluoroquinolones, trimethoprim-sulfamethoxazole and the aminoglycosides [5,12]. In our study ,we also found a high degree of resistance to the multiple classes of antibiotics among the ESBL producing isolates, which included the quinolones/fluoroquinolones: 82.5%, sulfonamide: 58.5%, aminoglycosides 55% and the β-lactam + β – lactamase inhibitor combinations: 19%-40%. Only the carbapenems group of antibiotics were the most active(11.50%) among all the antimicrobials which were tested.

Several studies have shown that the fluoroquinolones, cephalosporins, and the β – lactam + β lactamase inhibitor combinations were frequently recommended as an empirical therapy for the infections which were caused by *E.coli* [16-18]. In our study, we also found that the β lactam + β lactamase inhibitors (51%) were considered as the most reliable class of antibiotics for the treatment of the infections which were caused by the ESBL producing *E.coli*, while for the non ESBL producing *E.coli* , the cephalosporins were the most prescribed antibiotics (34%).

The outcome of our study indicated that 65.5% of the patients improved with the proper antibiotic treatment, whereas 15% patients developed re infections and 16% of the patients expired due to the infections caused by ESBL producing *E.coli*. The mortality was significantly higher among the patients with blood stream infections, which was comparable to the findings of previous studies [3,14,15]. The mortality is mainly caused by the ESBL producing ExPEC. In India, no studies have been done, which have described the clinical outcome of the ExPEC infection.

Carbapenam resistance was seen in nearly 8% of the patients, thus indicating that we do have infections which are caused by the much hyped superbug and that caution is needed in recognizing these infections and in using the appropriate antibiotics. However, we did not test for the metallo-β-lactamase production. The future studies should include this.

In conclusion, our study showed a high prevalence of the ESBL producing *E.coli* among the hospitalized patients .These infections are more severe and they are associated with sepsis and multi organ failure, leading to poor outcomes, with an increase in the mortality as well as the relapses. An appropriate empirical antibiotic therapy with either carbapenam or the β lactum + β lactamase inhibitor combination for an appropriate duration may prevent the morbidity and the mortality in these patients and it may also prevent the relapse of these infections. The carbapenamase producing *E. coli* infections are not uncommon in our setting and they are likely to increase, leaving very few choices for the clinicians who treat these infections. A rational antibiotic use, using an antibiotic policy, screening of the high risk patients for these infections at the earliest and getting an evidence of the infection by doing the appropriate cultures, will prevent the mortality and the morbidity in these patients.

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