

Original Research Article

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## Hemolysin and Serum Resistance among ESBL Producing Extraintestinal Pathogenic *Escherichia coli* Isolated from a Tertiary Care Hospital

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### ABSTRACT

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*Escherichia coli* represent a major cause of morbidity and mortality worldwide. The treatment of *Escherichia coli* infections is now threatened by the emergence of antimicrobial resistance. The dissemination of resistance is associated with genetic mobile elements such as plasmids that may also carry other virulence determinants. The present study was undertaken to detect the virulence factors and drug resistance of extraintestinal pathogenic *Escherichia coli*. A total of 300 Extraintestinal pathogenic *Escherichia coli* isolated from various clinical samples and 50 commensal *E. coli* isolated from apparently healthy individuals were screened for virulence factors like serum resistance and hemolysin production. All Extraintestinal pathogenic *Escherichia coli* isolates were studied for antibiotic susceptibility pattern by modified Kirby-Bauer disc diffusion method. Extended spectrum  $\beta$ -lactamase (ESBL) production was screened by standard disc diffusion method and confirmed by CLSI phenotypic confirmation test. Among the 300 extraintestinal pathogenic *Escherichia coli* isolates 85 (28.33%) were hemolytic, 126 (42%) were resistant to bactericidal action of serum and 154 (51.33%) produced ESBL. The present study highlights the expression of virulence factors and multiple drug resistance in extraintestinal pathogenic *Escherichia coli* and the judicial use of antibiotics for management of their infections.

### Introduction

*Escherichia coli* strains causing disease outside the gastrointestinal tract have been named Extraintestinal pathogenic *Escherichia coli* (ExPEC). Extraintestinal pathogenic *Escherichia coli* infections are more commonly associated with infections

facilities, and in hospitals. Urinary tract is the most frequent extra intestinal site, causing 85–95% of cases of uncomplicated cystitis and pyelonephritis. Pneumonia, neonatal meningitis, surgical site infections, abdominal and pelvic infections are other

extra intestinal types of infections (Gabriela et al., 2012, Smith et al., 2007).

The virulence potential of *Escherichia coli* is largely determined by the presence of specialized virulence factors (VFs) such as Haemolysin, Serum resistance, Cell Surface Hydrophobicity, Pili / Fimbriae (Type-1 fimbriae, P fimbriae, S fimbriae, PAP), K antigen, Somatic O antigen, fimbrial adhesions, Haemagglutination of erythrocytes, expression of Siderophore Aerobactin, production of Colicin V and Cytotoxic Necrotizing Factor (Blanc et al., 1996). The virulence traits of extraintestinal pathogenic *Escherichia coli* are distinct from those of intestinal pathogenic *Escherichia coli* and other gram negative bacilli that cause disease outside the bowel (Russo and Johnson., 2000). These virulence markers are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to urethritis, cystitis, pyelonephritis, bacteremia and septic shock.

Production of  $\beta$ -lactamases are the important mechanism of drug resistance among the Gram negative bacteria. Extended spectrum  $\beta$  lactamases (ESBLs) belong to group 2be of Bush's functional classification and are derived from the point mutation in original plasmid mediated TEM-1 and SHV-1  $\beta$  lactamases (Bush et al., 1995). By definition, ESBL producing organisms confer resistance to penicillin, cephalosporins and monobactams. However, they cannot hydrolyze cephamycins and are inhibited by Clavulanic acid (Paterson and Bonomo., 2005, Bradford PA.2001).

The objectives of the present study were to evaluate virulence factors (serum resistance and hemolysin production) of extraintestinal pathogenic *Escherichia coli* with commensal strains of *Escherichia coli* isolated from stool samples of healthy individuals and

antibacterial resistance pattern of extraintestinal pathogenic *Escherichia coli* with special reference to ESBL production.

## Materials and Methods

This study was conducted in the department of Microbiology, Hassan Institute Medical Sciences, Hassan, over a period three years. Ethical clearance has been obtained from the institution. A total of 300 extraintestinal pathogenic *Escherichia coli* (test) isolated from clinical specimens and 50 commensal *Escherichia coli* isolated from feces of normal healthy individuals formed the study material.

Identification of the isolates was done on the basis of the colony morphology, Gram staining and the standard biochemical tests (John et al., 1994). Antibiotic sensitivity test (Kirby-Bauer disk diffusion method) was done by using Amikacin(30  $\mu$ g), amoxicillin-clavulanic acid(20/10 $\mu$ g), ampicillin(10 $\mu$ g), ceftioxone (30 $\mu$ g), cefodoxime(10 $\mu$ g), cefotaxime (30 $\mu$ g), cefoxitin (30 $\mu$ g), ceftazidime(30 $\mu$ g), ceftazidime-clavulanic acid(30/10  $\mu$ g), ciprofloxacin (5 $\mu$ g), cotrimoxazole (25 $\mu$ g), gentamicin (10 $\mu$ g), imepenem(10 $\mu$ g), norfloxacin (10 $\mu$ g), discs (HiMedia) CLSI,2006).

## Detection of Serum Resistance

Serum resistance was studied using fresh culture of the isolates grown at 37 °C on blood agar. The harvested cells were suspended in Hank's balanced salt solution (HBSS). Bacterial suspension (0.05 mL) was incubated with serum (0.05 mL) at 37°C for 180 min. The control wells contained bacterial suspension (0.05 mL) and equal volume of HBSS. 10  $\mu$ l of samples were withdrawn at 0 minute and after 180 minutes of incubation and spread

on blood agar plates which were then incubated at 37 °C for 18 h and the viable count was determined. Resistance of bacteria to serum bactericidal activity is expressed as the percentage of bacteria surviving after 180 min of incubation with serum, in relation to the original count. The bacteria were termed serum sensitive, if viable count dropped to 1% of initial value and resistant, if >90% of organisms survived after 180 minutes (Raksha et al., 2003).

### **Detection of Hemolysis**

Plate hemolysis test was done by using 5% sheep blood agar for the detection of  $\alpha$ -hemolysin produced by *Escherichia coli*. *Escherichia coli* isolates were inoculated onto sheep blood agar and incubated overnight at 35 °C. Presence of a zone of complete lysis of the erythrocytes around the colony and clearing of the medium indicates haemolysin production (Siegfried et al., 1994).

### **Detection of ESBL Producers**

#### **CLSI Phenotypic Confirmation Test**

*Escherichia coli* strains resistant to Ceftazidime and Cephotaxime in antibiotic sensitivity test were subjected to phenotypic confirmation test for ESBL. In a lawn culture of the strain, Ceftazidime (30 $\mu$ g) Vs Ceftazidime- Clavulanic acid (30/10 $\mu$ g) and Cephotaxime (30 $\mu$ g) Vs Cephotaxime-Clavulanic acid (30/10 $\mu$ g), discs were placed and incubated at 37°C overnight.

Regardless of zone diameter, a  $\geq$  5mm increase in zone diameter of the Cephalosporin tested in combination with Clavulanic acid Vs its zone size when tested alone was taken as ESBL producer. *Escherichia coli* ATCC 25922 was used as the negative control and ESBL - producing organism *Klebsiella pneumoniae* ATCC

700603 was used as the positive control (CLSI, 2006).

### **Results and Discussion**

Out of 300 extraintestinal pathogenic *Escherichia coli* 167 were from urine, 81 from pus, 26 from sputum, 12 from blood and 14 from endotracheal tubes + catheter tips. All the isolates (ExPEC and Control) were studied for hemolysin production and serum resistance. All ExPEC isolates were subjected to antibiotic sensitivity test. ESBL production was detected by screening test and phenotypic confirmation test.

Among 300 extraintestinal pathogenic *Escherichia coli* 126 (42%) showed resistance to bactericidal action of serum. Highest serum resistance was seen in isolates from blood 10(83.33%) followed by endotracheal tubes 10 (71.42%), sputum 14(53.84%), pus 39 (48.14%) and urine 53 (31.73%). Out of the 50 commensal *Escherichia coli* 10 (20%) exhibited resistance to bactericidal action of serum. A previous study conducted by Sharma et al (2007), showed serum resistance in 77.4% of *Escherichia coli* isolated from blood which is similar to our results. In another study 32.7% of *Escherichia coli* were resistant to bactericidal action of serum (Raksha et al.,2003). However, Kauser et al (2009), Prachi et al (2012), Sabitha Baby et al (2014),and Siegfried et al (1994) have reported higher serum resistance in uropathogenic *Escherichia coli*. Bacterial resistance to killing by serum results from individual or combined effects of capsular polysaccharide and surface proteins (Taylor., 1983).

Hemolysin production is a property associated with *Escherichia coli* strains that infect extraintestinal sites in humans, where as it is rarely found in fecal isolates from healthy individual (Caprioli A, 1989). In the

present study, hemolysin production was observed in 85 (28.3%) extraintestinal pathogenic *Escherichia coli* and 7 (14%) commensal *Escherichia coli* isolates respectively. Hemolysin production was highest among the isolates of endotracheal tubes + catheter tips ie, 7(50%) followed by blood 5 (41.6%), urine 51 (30.53%) pus 17

(20.98%) and sputum 5(19.2%).

Sharma et al (2007) reported hemolysin production in 64.2% of *Escherichia coli* isolated from blood. Raksha et al (2003), Johnson (1991) and Fakrunddin et al (2013) have reported highest hemolysin production among urinary isolates.

**Table.1** Virulence Markers of Extra Intestinal Pathogenic and Commensal *Escherichia coli* Isolates

Virulence factors	Urine (167)	Pus (81)	Sputum (26)	Blood (12)	catheter tips(14)	Total (300)	Control (50)	p
Serum resistance	53 (31.73%)	39 (48.1%)	14 (53.8%)	10 (83.3%)	10 (71.4%)	126 (42%)	10 (20%)	< 0.05
Haemolysin	51 (30.53%)	17 (20.98%)	05 (19.2%)	05 (41.6%)	07 (50%)	85 (28.3%)	07 (14%)	<0.05

**Table.2** ESBL Production in Extra Intestinal Pathogenic *Escherichia coli*

Sample	Number of isolates	ESBL positive	Percentage
Urine	167	86	51.49
Pus	81	38	46.91
Blood	12	7	58.33
Sputum	26	11	42.3
Catheter tips	14	12	85.71
Total	300	154	51.33

**Table.3** ESBL Production by Serum Resistant and Serum Sensitive Extraintestinal Pathogenic *Escherichia coli*

Serum resistant(126)			Serum sensitive (174)			p
ESBL Positive	ESBL Negative	Percentage	ESBL Positive	ESBL Negative	Percentage	
65	61	51.58	89	85	51.14	> 0.05

**Table.4** ESBL Production by Haemolytic and Non Haemolytic Extraintestinal Pathogenic *Escherichia coli*

Haemolytic(85)			Non Haemolytic(215)			p
ESBL Positive	ESBL Negative	Percentage	ESBL Positive	ESBL Negative	Percentage	
52	33	61.17	102	113	47.44	< 0.05

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Antibiotic susceptibility pattern was studied for all isolates of extra intestinal pathogenic *Escherichia coli*. Resistance was observed to commonly used antibiotics such as ampicillin, ciprofloxacin, co-trimoxazole, cefotaxime, gentamicin, nalidixic acid, ceftriaxone and ceftazidime where as all the strains were susceptible to Imepenem. In the present study we found that 76.9% isolates were resistant to ampicillin. These results were consistent with the previous studies on drug resistance in *Escherichia coli* (Gupta et al., 1999, Suman and Bhat GK 2001). The greater prevalence of resistance to common antibiotics has also been reported by other workers (chitins et al., 2003 and Weiner et al 1993). Several bacteria, including *Escherichia coli*, construct a multiple-antibiotic-resistance efflux pump that provides the bacterium with resistance to multiple types of antibiotics, including erythromycin, tetracycline, ampicillin and nalidixic acid. This pump expels the antibiotic from the cell's cytoplasm, helping to maintain the intracellular levels below a lethal concentration. (Oethinger M.et al., 1988).The higher rates of multidrug resistance in *Escherichia coli* may be related to the dissemination of antibiotic resistance among hospital isolates of *Escherichia coli*.

*Escherichia coli* is the most common ESBL producing Gram negative bacteria isolated from different clinical samples followed by *Klebsiella*. The present study detected 51.3% of ESBL production among ExPEC and results of the present study correlate with other studies from Karnataka. (Ananthakrishna et al., 2000, Nair T Bhaskaran et al., 2011). In the present study, the highest percentage of ESBLs was observed among inpatients (77.2%). In 2006, the Antimicrobial Availability Task

Force of the Infectious Diseases Society of America listed ESBL-producing Enterobacteriaceae (*Klebsiella* species and *Escherichia coli*) as one of six problematic drug-resistant pathogens and suggested an urgent need for newer and more effective therapeutics (Talbot et al., 2006). The high rate of ESBL production by extra intestinal pathogenic *Escherichia coli* may be due to the selective pressure imposed by extensive use of antimicrobials and indiscriminate use of cephalosporins. In infections with ESBL producing strains, a slight increase in MICs of oxyimino-cephalosporins has been reported to be sufficient to cause treatment failure. The availability of antibiotics without a prescription, their widespread use by the general public often with suboptimal dosing and duration of therapy, contribute to the emergence of this problem. The judicious use of antibiotics, good antibiotic policy, periodic surveillance of antibiotic resistance patterns and accurate detection and reporting of ESBL production by pathogenic bacteria are needed to limit the emergence and spread of antibiotic resistance in bacteria.

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