



Original Research Article

Study of virulence factors and antibiotic susceptibility pattern of extraintestinal pathogenic *Escherichia coli*

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ABSTRACT

Aims: To detect the Virulence factors of extraintestinal pathogenic *Escherichia coli* (ExPEC). To study the antimicrobial susceptibility pattern and Extended spectrum Beta lactamase (ESBL) production by ExPEC.

Settings and Design: This is a prospective study conducted on *Escherichia coli* (*E coli*) isolated from extraintestinal sites.

Materials and Methods: A total of 150 ExPEC isolates were obtained from various clinical samples. These isolates were tested for production of Hemolysin, Hemagglutination, Serum resistance and Gelatinase. Isolates were also tested for susceptibility to a set of antimicrobials and for production of an enzyme Extended spectrum Beta lactamase (ESBL).

Statistical Analysis: Statistical analyses were done with IBM SPSS Version 22. A “P” Value of <0.05 was considered as significant.

Results: Among 150 isolates of *E coli*, 53 (35.3%) were haemolytic, 66 (44%) were positive for serum resistance, 77 (51.3%) were positive for gelatinase and 78 (52%) were positive for Hemagglutination. Antibiotic susceptibility pattern showed that maximum isolates (~90%) were resistant to Cephalosporins and Ampicillin. Least resistance (13.1%) was shown to Nitrofurantoin in cases of isolates from urine sample. Testing for ESBL production showed that majority (60.7%) of the isolates were confirmed to be ESBL producers.

Conclusion: *E coli* cause various extraintestinal infections because of production of virulence factors & developing drug resistance. Judicious use of antibiotics can prevent drug resistance.

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1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains are *Escherichia coli* (*E coli*) strains which can cause infection outside the intestinal tract. This is because of expression of Virulence factors (VFs) which are Hemolysin, Hemagglutination, Serum resistance, Gelatinase production, Resistance to phagocytosis, Cell surface hydrophobicity, Siderophore production, etc.¹ Because of these virulence factors, microorganism colonize a host niche and cause tissue damage.²

Also they have developed resistance to numerous antimicrobials. Multidrug resistance is because of production of enzymes like Extended spectrum Beta lactamase (ESBL) which has lead to limited therapeutic options.³

2. Materials and Methods

It is a prospective study which was conducted on ExPEC isolates obtained from various clinical samples other than stool sample between October 2015 to March 2017. Isolates were identified based on microscopy, culture characteristics & standard biochemical tests.^{4,5} Antimicrobial susceptibility testing was done for all the isolates using Kirby-Bauer’s disc diffusion method using

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Clinical and laboratory standards institute (CLSI) guidelines 2017.⁶ ESBL production was screened phenotypically by an initial screening test, which was followed by confirmatory double disk synergy test set by CLSI guidelines 2017.⁶

Statistical analysis: Statistical analyses were done with IBM SPSS Version 22 for Windows.

A “P” Value of <0.05 was considered as significant.

2.1. Detection of virulence factors

HEMOLYSIN: The *E coli* isolates are inoculated on 5% sheep blood agar and incubated overnight at 37°C. The indicator of Hemolysin production is the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.^{7,8}

2.2. Hemagglutination

The test is carried out as per the direct bacterial Hemagglutination test- slide method. One drop of red blood cell (RBC) suspension is added to a drop of broth culture and the slide is rocked at room temperature for five minutes. Presence of clumping is taken as positive for Hemagglutination. Mannose sensitive Hemagglutination (MSHA) is detected by the absence of Hemagglutination in a parallel set of test in which a drop of 2% W/V D-Mannose is added to the red cells and a drop of broth culture. Mannose resistant Hemagglutination (MRHA) is detected by the presence of Hemagglutination of 3% ‘O’ blood group human RBCs in the presence of 2% W/V D-Mannose.⁹

2.3. Serum resistance

Overnight culture of *E coli* on blood agar plates are suspended in hank’s balanced salt solution. Equal volume of this bacterial suspension and serum (0.05ml) are incubated at 37°C for three hours. Then 10µl of this mixture is inoculated on blood agar plate and incubated at 37°C for 24 hours and viable count is determined. It is termed as sensitive if viable count drops to 1% of initial value and resistant if 90% of the organisms survived after 180 minutes.^{7,8}

2.4. Gelatinase test

Gelatinase production is tested using gelatin agar. The plate is inoculated with test organism and incubated at 37°C for 24 hours. The plate is then flooded with 1% Tannic acid solution. Development of opacity around colonies is considered as positive for gelatinase.¹⁰

3. Result

Age distribution of isolates showed that maximum isolates were obtained from age group 20-29 years which was 34 (22.7%). Gender distribution showed that the number of isolates obtained from male and female were almost same

with 77 (51.3%) isolates from male patients and 73 (48.7%) isolates from female patients with ratio of 1:1.

Table 1: Specimen wise distribution of *E coli* isolates

Nature of Specimen	Frequency	Percentage
Urine	84	56.0
Pus	38	25.3
Blood	12	8.0
Sputum	7	4.7
Endotracheal secretion	4	2.7
Others	5	3.3
Total	150	100.0

Maximum isolates were obtained from urine sample 84 (56%) & minimum isolates from endotracheal secretions 4 (2.7%) [Table 1].

Table 2: Frequency of various virulence factors

Virulence factors	Frequency	Percent
Hemolysin	53	35.3
Serum resistance	66	44.0
Gelatinase	77	51.3
Hemagglutination	78	52

Of all the virulence Factors, most common was Hemagglutination 78 (52%), followed by gelatinase 77 (51.3%), serum resistance 66 (44%) and Hemolysin 53(35.5%) [Table 2].

Of the isolates positive for Hemagglutination 35 (23.3%) isolates were positive for MRHA and 43 (28.6%) isolates were positive for MSHA.

Table 3: Antibiotic resistance pattern

Antibiotics	Frequency (N=150)	Percent
Ampicillin	135	90.0
Cefazolin	136	90.7
Gentamicin	84	56.0
Tetracycline	60	40.0
Ciprofloxacin	114	76.0
Ceftazidime	132	88.0
Cefotaxime	129	86.0
Cefpodoxime	121	80.7
Ceftriaxone	135	90.0

Antibiotic resistance pattern showed that maximum isolates (~90%) were resistant to cephalosporins and ampicillin. Least resistance (40%) was shown to tetracycline [Table 3].

Resistance pattern for norfloxacin and nitrofurantoin among *E coli* isolated from urine samples showed that 78.6% of the isolates were resistant to norfloxacin and least resistance (13.1%) was shown to nitrofurantoin [Table 4].

ESBL production showed that 91 (60.7%) of the isolates were confirmed to be ESBL producers [Table 5].

Table 4: Resistance pattern for norfloxacin & nitrofurantoin

Urine sample (N=84)		
Antibiotics	Resistant	
	Frequency	Percent
Norfloxacin	66	78.6
Nitrofurantoin	11	13.1

Table 5: ESBL production

ESBL	Resistant	
	Frequency (N=150)	Percent
Positive	91	60.7
Negative	59	39.3

ESBL: Extended spectrum beta lactamase

Table 6: Correlation between ESBL production and production of different virulence factors

ESBL Production	Virulence factors (Positive)		
	Hemolysin	Serum resistance	Hemagglutination
Positive	34	17	19
Negative	19	25	31

ESBL: Extended spectrum beta lactamase

Hemolysin production was more among ESBL positive strains when compared to ESBL negative strains. Whereas Serum resistance and Hemagglutination was seen more among ESBL negative strains compared to ESBL positive strains [Table 6].

4. Discussion

ExPEC strains are distinct from most intestinal commensal *E coli* as well as from diarrheagenic *E coli* types.¹¹ These strains are distinct by the production of virulence factors. These virulence factors are encoded by genes which are arranged in large blocks known as pathogenicity islands. They can be horizontally transferred among distinct *E coli* strains causing various extraintestinal infections.¹²

Along with these virulence factors, these strains have developed resistance to several antibiotics leading to difficulties in treatment.¹³ Multidrug resistance is because of production of enzyme ESBL. The incidence of ESBL producing *E coli* strains is increasing resulting in limited therapeutic options.¹⁴

Urinary tract infection (UTI) is one of the major extraintestinal infections caused by *E coli*. In the present study 56% of the isolates were from urine sample. This is similar to a study conducted by Shenoy et al. (2014)¹³ where 65% of the isolates were obtained from urine sample.

Hemolysin is toxic to host cells by contributing to inflammation & tissue injury.¹⁵ Haemolytic strains are mainly associated with UTI. In the present study 35.3% isolates were positive for hemolysin. This is similar to the

study conducted by Shenoy et al (2014)¹³ where hemolysin production was seen in 34% of the isolates.

Agglutination of RBCs is an indirect evidence of presence of fimbriae. P fimbriae, Dr fimbriae, X, FIC are responsible for MRHA.¹⁶ It plays an important role in adhesion and establishment of pathogenic strains to various host tissues.¹⁷ This is important especially in renal system where adhesion and colonization of urinary tract can lead to UTI. In the present study Hemagglutination was seen in 52% of the isolates with MRHA being 23.3% and MSHA 28.6%. Similar observation was made in Kauser Y et al. (2009)¹⁸ where MRHA & MSHA production was seen in 30% & 36% of the isolates respectively.

Serum resistance is a property of bacteria by which it resists killing by normal serum. It is individual or combined effect of outer membrane protein (OMP), capsular polysaccharide & somatic antigens. In the present study 44% isolates were resistant to serum. This is similar to the study conducted by Shetty et al. (2014)¹ where serum resistance was seen in 45.33% of the isolates.

Gelatinase, an important virulence factor which is capable of hydrolyzing gelatin, collagen, and is associated with inflammation. Shetty et al. (2014)¹ observed that gelatinase is not an important virulence factor. While Mittal et al. (2014)¹⁹ observed that gelatinase producing strains were multidrug resistant. In the present study Gelatinase production was seen in 51.3% of isolates which is similar to Mittal et al. (2014)¹⁹ where it was 67.5%. But Vaish et al. (2016)²⁰ & Jayanthi et al. (2017)²¹ showed lesser production of gelatinase which was 2% & 6% respectively.

The present study showed >80% of the isolates were resistant to Ampicillin and Cephalosporins. Kauser Y et al. (2009)¹⁸, Vaish et al. (2016)²⁰ & Jayanthi et al. (2017)²¹ also showed 70-90% of resistance to Ampicillin & Cephalosporins. Resistance to commonly used antibiotics is because of excessive use and misuse of the antibiotics by the healthcare personnel and dissemination of multidrug resistance among hospital strains.^{22,23}

In the present study resistance to Nitrofurantoin and Norfloxacin was 13% and 78.6% among isolates obtained from urine sample. Similar results were shown by Kauser Y et al (2009)¹⁸ where resistance to Nitrofurantoin and Norfloxacin was 15% & 85% respectively. Nitrofurantoin is one of the old urinary antibiotics. Resistance to this drug is minimal in the present as well as past studies. The reason may be because of its limited usage in other infections or because of multiple mechanisms of resistance, requiring organisms to develop more than a single method of resistance.

In the present study 60.7% of the isolates produced ESBL. Sharma et al. (2007)²⁴ observed ESBL production in 52% of the isolates. High rate of ESBL production may be because of selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is

responsible for the high rate of selection of ESBL producing microorganisms.^{25,26}

Correlation between ESBL production and production of different virulence factors showed that Hemolysin production was more among ESBL positive strains when compared to ESBL negative strains. Whereas Serum resistance and Hemagglutination was more among ESBL negative strains compared to ESBL positive strains. This indicates that there is a negative correlation between ESBL production and production of different virulence factors. This is because although virulence factors and antibiotic resistance may confer increased fitness for extraintestinal infections in humans, they may do it via mutually exclusive pathways and in distinct populations.²⁶

5. Conclusion

E.coli adapt and survive in different tissues because of their ability to produce virulence factors and by developing drug resistance. Various virulence factors enable organisms resist phagocytosis, adhere and colonize tissues, lyse RBCs and WBCs, injure tissues, grow in tissues deprived of iron, resist bactericidal action of serum etc. Along with this, by producing enzymes like ESBL and Metallo β lactamase (MBL), these strains have developed resistance to multiple drugs. Therefore judicious use of antibiotics and good antibiotic policy are important to limit the emergence and spread of antibiotic resistance in bacteria.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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