IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 3 Ver. V (Mar. 2015), PP 15-21
www.iosrjournals.org

Co-Relation between Virulence Factors and Antibiotic Resistance of *E. coli*, With Special Reference to Uropathogenic *E. coli*

Sneha Kukanur[1], Meera Meundi[2], Ashish Bajaj[1], Subbannayya Kotigadde[3]
[1]Post graduate, [2]Professor & Head, [3]Professor, Department of Microbiology, KVG Medical College & Hospital, Sullia, DK, Karnataka, India.

Abstract: *Escherichia coli is a part of normal intestinal flora. When it enters into unnatural sites, it can cause infections such as urinary tract infections (UTI), wound infections, lower respiratory tract infections (LRTI) etc. This is achieved by some virulence factors such as production of beta-haemolysin, biofilm & extended spectrum beta lactamase (ESBL) which endow its ability to survive, multiply & cause disease. This study was done to isolate E. coli from extraintestinal clinical samples, to assess their virulence factors such as β-haemolysin & biofilm production and to determine antibiotic susceptibility pattern & ESBL production by phenotypic method.*
A total of 52 E. coli isolates were obtained from urine(28), pus(22) & sputum(2) samples. All isolates were identified as per standard procedures. Beta-haemolysis using 5% sheep blood agar & biofilm production by tube method were determined. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method. ESBL production was evaluated by screening and phenotypic double disc synergy test as per CLSI guidelines. Out of 52 isolates, 13(25%) produced Beta-haemolysis of which 8(61.5%) were from urine samples. 38(73%) produced biofilms of which 21(55.2%) were from urine samples. 28(53.8%) produced ESBL of which 14(50%) were from urine samples. Most isolates were sensitive to amikacin and resistant to cefotaxime.
It concludes that biofilm producing organisms are difficult to treat as they are resistant to commonly used antibiotics. Evaluation of Beta-hamolysin, biofilm and ESBL production should be employed in routine evaluation of E. coli isolates for early detection and prompt treatment.

Keywords *–Beta haemolysis, Biofilm, Extended spectrum beta lactamase, Escherichia coli.*

I. Introduction

Escherichia coli comprises of non-pathogenic commensal isolates that forms part of the normal flora of humans and various animals. However, several variants have been described that cause infection of the gastrointestinal system (intestinal pathogenic *E. coli*) while others cause infections outside the gastrointestinal system (extraintestinal pathogenic *E. coli* or ExPEC).

Urinary tract infection (UTI), sepsis, and neonatal meningitis are the most studied extraintestinal infection syndromes caused by ExPEC. The causative strains constitute a distinctive subset of the *E. coli* population characterized by a diverse virulence factors (VFs), such as adhesins, iron sequestration systems, toxins, and polysaccharide coatings.[1,2]

Urinary tract infections are probably the most common bacterial infections.[3] The primary reservoir of UPEC is believed to be the human intestinal tract and employ diverse repertoire of virulence factors to colonize and infect the urinary tract in an ascending fashion.[2] Manifestations can vary from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and blood stream infection. A single bacterial species, *Escherichia coli*, causes majority of UTI.[3]

Haemolysin provides *E.coli* with possible selective advantage by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic and epithelial cells.[3] Haemolytic *E.coli* are more likely to cause disease than non-haemolytic *E.coli*.[4]

Relentless use of β‑lactam antibiotics in the clinical practice has resulted in the appearance of newer β‑lactamases such as extended spectrum β‑lactamases (ESBLs), are typically plasmid mediated and seen mainly in *Escherichia coli* and *Klebsiella pneumoniae*.[5]

Biofilms are a group of microbes which are encased in an exopolysaccharide matrix on both biotic and abiotic surfaces. This causes a number of persistent infections which respond poorly to conventional antibiotic therapy.[6] Some strains of *E. coli* also possess the ability to produce biofilms and are being resistant to wide range of commonly used antibiotics.[7]

Measuring a phenotype in-vitro does not always correlate with in-vivo expression and may underestimate the presence of a virulence factor invivo. Identifying a genotype, on the other hand, does not mean that it is expressed in the body.[3] However, there is need to identify, prevent and treat all the infections caused by ExPEC efficiently in order to reduce the incidence of this multidrug resistance highly virulent superbug.

II. Material And Methods

The study was conducted at the Department of Microbiology K.V.G. Medical College and Hospital, Sullia. A total of 52 isolates of *E. coli* were isolated from various clinical samples such as urine, sputum and pus received for routine laboratory investigations from patients attending to the hospital over a period of three months. The ethical clearance for the conduction of the study was obtained from Institutional Ethical Committee. These isolates were identified as *E. coli* by conventional methods.

Beta haemolysin production was demonstrated by using 5% sheep blood agar. Complete haemolysis around the colonies was taken as positive.[3]

Biofilm production was done by a qualitative Tube method. A loopful of test organism was inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37^0C for 24 hours. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.[7]

The antimicrobial susceptibility testing of these isolates was performed on Mueller-Hinton agar (MHA) using commercial antibiotic discs (Hi-Media Laboratories Ltd, Mumbai, India) by the standard Kirby-Bauer disc diffusion method and interpreted as per CLSI recommendation.[8] The antibiotics used were amoxyclav (30µg), nalidixic acid (30µg), nitrofurantoin (300µg), gentamicin (10µg), amikacin (30µg), norfloxacin (10µg), sparfloxacin (5µg), co-trimoxazole (25µg), cefotaxime (30µg) and ceftriaxone (30µg). *E. coli* ATCC 25922 was used as control.

ESBL detection: Isolates that showed resistant to at least one of the third generation cephalosporins (cefotaxime, ceftriaxone) were tested for ESBL production by both double disc synergy test and phenotypic confirmation test recommended by CLSI.

Double disc synergy test (DDS): This test was performed as described by Jarlier et al. with some modifications. The standard inoculum of the isolates (McFarland 0.5 standard) was lawn cultured onto MHA plate. Ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg) and cefpodoxime (10µg) discs were placed at 30mm distance (centre to centre) from amoxicillin/ clavulanate disc (20/10µg). A clear extension of the edge of the antibiotic zone of inhibition towards amoxicillin/clavulanic acid disc indicated ESBL production.[8]

CLSI phenotypic confirmation test: A McFarland 0.5 standard suspension of the isolate was inoculated onto MHA plate. Ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg), cefotaxime (30µg) and cefotaxime/clavulanic acid (30/10µg) discs were placed on inoculated MHA plate at a distance of 30mm apart from centre to centre. The culture was incubated at 37°C overnight. The observation of a ≥5mm increase with ceftazidime/clavulanic acid and cefotaxime/clavulanic acid than ceftazidime and cefotaxime alone was considered ESBL producer.[9]

III. Results

Of the 52 isolates of *E. coli*, 28(53.8%) were isolated from urine, 22(42.3%) from pus and 2(3.8%) from sputum samples. 38 (73%) were in patients and 14 (26.9%) were out patients. (Table 1)

Table 1: Distribution of *E. coli* among different clinical samples.

Type of sample	Number of samples	OP	IP
Urine	28 (53.8%)	12	16
Pus	22 (42.3%)	2	20
Sputum	2 (3.8%)	0	2
Total	52 (100%)	14 (26.9%)	38 (73%)

Among the 52 samples of *E. coli* obtained, most of them were from the department of surgery followed by medicine, obstetrics and gynaecology, orthopaedics and paediatrics. (Table 2)

Table 2: Distribution of *E. coli* among various departments.

Department	No. of samples
Medicine	17 (32.6%)
Surgery	28 (53.8%)
Orthopaedics	2 (3.8%)
OBG	3 (5.7%)
Paediatrics	2 (3.8%)

Most of the isolates were from the age group 51-60 years (30.7%) followed by 41-50 years (28.8%) and 61-70 years (21.1%). In our study group there was a slight male preponderance and their strength being 29 (55.7%). (Table 3)

Table 3: Distribution of age and sex among different samples.

Age (years)	Urine (28)	Pus (22)	Sputum (2)	Total (52)	Male (29)	Female (23)
0-10	2 (7.1%)	-	-	2 (3.8%)	1	1
11 to 20	-	-	-	-	-	-
21 to 30	1 (3.5%)	2 (%)	-	3 (5.7%)	2	1
31 to 40	1 (3.5%)	1 (%)	-	2 (3.8%)	-	2
41 to 50	4 (14.2%)	10 (%)	1 (50%)	15 (28.8%)	9	6
51 to 60	8 (28.5%)	2 (%)	1 (50%)	11 (30.7%)	7	4
61 to 70	5 (17.8%)	6 (%)	-	11 (21.1%)	6	5
71 to 80	5 (17.8%)	1 (%)	-	6 (11.5%)	3	3
81 to 90	2 (7.1%)	-	-	2 (3.8%)	1	1

Figure 1: Biofilm production – Tube method.

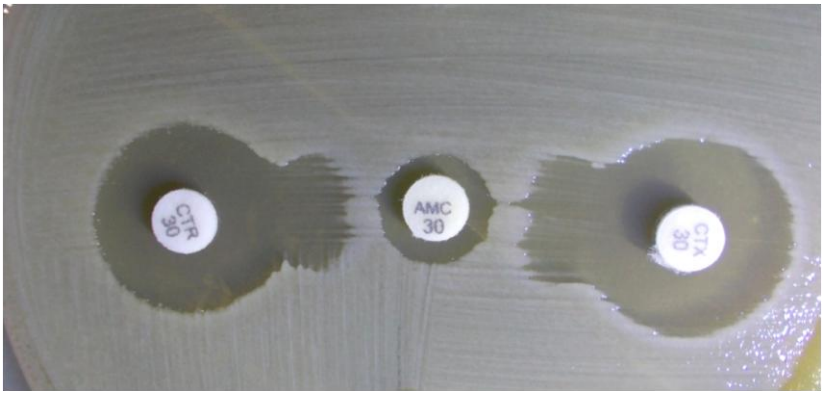

Figure 2: ESBL detection – Double disk synergy test.

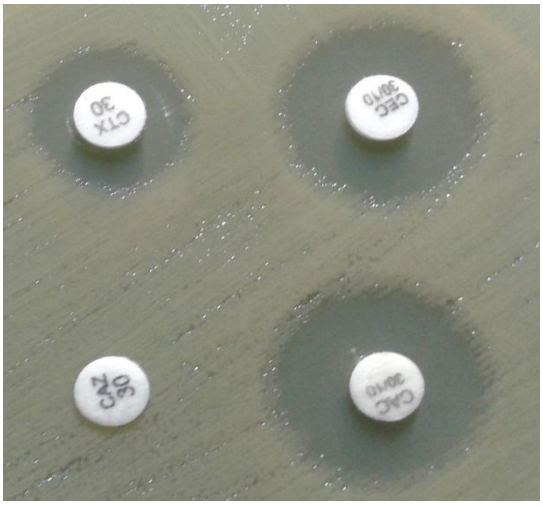

Figure 3: ESBL detection - CLSI phenotypic confirmation test.

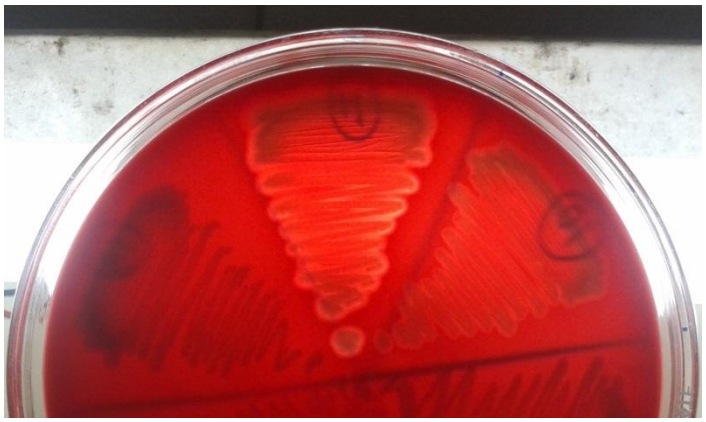

Figure 4: Beta haemolysis on 5 % sheep blood agar.

Table 4: Distribution of beta haemolysis, biofilm formation and ESBL production among different clinical samples.

Type of sample	Biofilm (38)	ESBL (27)	Beta hemolysis (13)
Urine	20 (52.6%)	14 (51.8%)	8 (61.5%)
Pus	17 (44.7%)	12 (44.4%)	3 (23%)
Sputum	1 (2.6%)	1 (3.7%)	2 (15.3%)

As the above table 4 shows among all the three virulence factors, most of the isolates were positive for bifilm formation 38 (73%) followed by ESBL production 27 (51.9%) and beta haemolysin production 13 (25%). Out of all 38 biofilm producing isolates 20 (52.6%) were from urine, 17 (44.7%) from pus and 1 (2.6%) were from sputum samples.(Fig. 1) Among all 27 ESBL producers isolated, 14 (51.8%), 12 (44.4%) and 1(3.7%) were from urine, pus and sputum samples respectively.(Fig. 2 & 3) Out of 13 beta haemolysin producers 8 (61.5%), 3 (23%) and 2(15.3%) were from urine, pus and sputum respectively.(Fig 4)

Table 5: Distribution of all 3 virulence factors in various combinations.

Virulence Factors	Urine	Pus	Sputum
Beta hemolysis and Biofilm production	7	2	1
Biofilm and ESBL production	9	5	1
ESBL and Beta hemolysis	6	1	1
Beta hemolysis, Biofilm and ESBL production	5	1	1

All the three virulence factors were observed in 7 *E. coli* isolates, of which 5 were from urine, 1 each was from pus and sputum. Most common combination of virulence factors was biofilm and ESBL production, which was observed in 17 isolates of which 9 isolates were from urine sample. (Table 5)

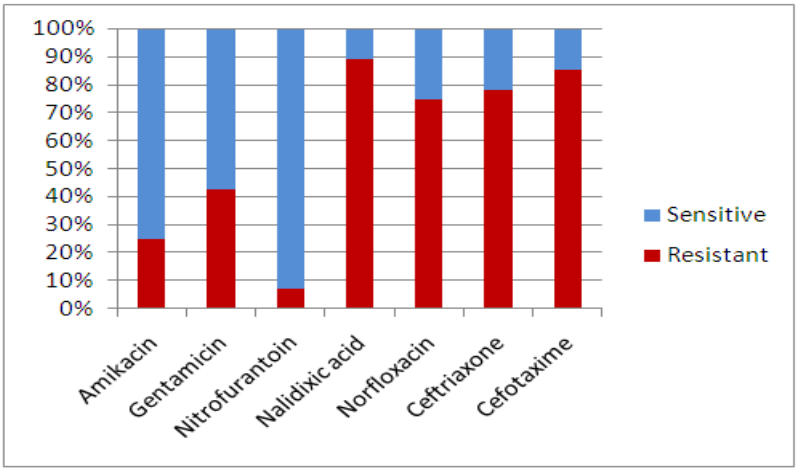

Figure 5: Antibiogram of Urine samples (n=28).

The antimicrobial susceptibility of 28 urine isolates was 92.5% to nitrofurantoin followed byamikacin (75%) and gentamicin (57.1%). Isolates were 89.2% resistant to nalidixic acid, followed by cefotaxime (85.7%), ceftriaxone (78.5%) and norfloxacin (75%). (Fig. 5)

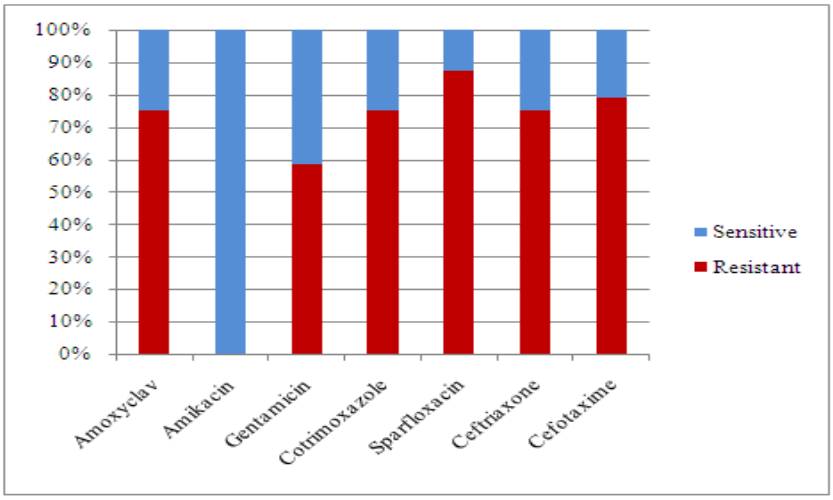

Figure 6: Antibiogram of pus(22) and sputum(2) samples.

The antimicrobial susceptibility of 24 pus and sputum isolates was 100% to amikacin. Isolates were87.5% resistant to sparfloxacin followed by cefotaxime (79.1%), ceftriaxone (75%), cotrimoxazole (75%) and amoxyclav (75%).(Fig. 6)

IV. Discussion

As UTI is the most common form of extra-intestinal *E. coli* infection,[1] in the present study we isolated more than 50% of *E. coli* from urine samples.

Our study samples obtained were more in the age group 41-70 years since,etiology of these infections is affected by underlying host factors that complicate the infection, such as diabetes, spinal cord injury, catheterization, benign prostatic hypertrophy, genetic factors, ageing, the menopause, urogenital dysfunction, sexual behavior, and previous pelvic surgery.[10]

There was a slight male preponderance, may be because we have not only taken urine samples but other extra intestinal samples like pus and sputum also into consideration.

73% of our cases were from in patient department suggesting that these may be the nosocomial isolates. 53.8% of our cases were from surgery department.

Out of 52(100%) isolates 38 (73%), 27(51.9%) and 13(25%) isolates showed biofilm, ESBL and Beta hemolysin production respectively. In a study from Himachal Pradesh, 10.24% of *E. coli* isolates were haemolytic,[4] whereas we isolated 25% of haemolytic *E. coli* of which 15.3% were from urine isolates. The haemolytic activity was more frequent among urinary strains than among other strains. Thus, haemolytic activity act as one of the virulence factors for pathogenesis of UTI.[4]

73% of our isolates were biofilm producers of which 38.4% were uropathogens. Biofilm production is one of the several putative virulence determinants possessed by UPEC. It is a dynamic process that can bring about wide variety of physiological events like antibiotic tolerance, expression of virulence factors and increased resistance to host defence mechanisms. Studies have shown that isolates collected from urine had a greater capacity to form in-vitro biofilm than those collected from other isolates. Uropathogenic *E. coli* forms biofilm-like structures on and inside host cells and the ability to form biofilms has been related to persistence of bacteria in the urinary tract.[11]

We reported 51.9% ESBL producers of which 26.9% were from urine samples. A report from Coimbatore (India) showed that ESBL production was 41% in *E. coli*. In a similar study by Mathur et al, showed 62% of *E. coli* to be ESBL producers.[12]

Our study revealed that 92.5%, 75% and 57.1% of urine isolates were sensitive to nitrofurantoin, amikacin and gentamicin respectively. Among pus and sputum samples amikacin was highly sensitive (100%). A study from CMC Vellore, India, showed that older drugs like nitrofurantoin appeared to be useful and could be considered as a choice for treating uncomplicated lower urinary tract infections. Aminoglycosides appeared to be best suited for complicated infections.[3] A study done by Umadevi et al, reported that amikacin showed good activity against gram-negative bacteria. Co-resistance to amoxicillin-clavulanate, gentamicin and ciprofloxacin was very common.[12] The ESBL-producing organisms are a breed of multidrug-resistant pathogens. It is essential to report ESBL production along with the routine sensitivity reporting, which helps the

clinicians in prescribing proper antibiotics. Piperacillin-tazobactam and imipenem are the most active and reliable agents for the treatment of infections which are caused by ESBL producing organisms.[12] However, the carbapenems are antimicrobials that are usually kept in reserve. In the case of non life-threatening infections and in non-outbreak situations, it is not necessary to administer carbapenems. This approach intends to preserve the therapeutic value of these precious drugs.[13] A study from Bangladesh stated that, uncontrolled consumption of the common antibiotics during the past decade influenced the spreading of resistance property among the causative agents. Inappropriate antibacterial treatment and abuse of antibiotics have also contributed to the emergence of antibacterial-resistant bacteria. Self prescription of antibacterials is an example of antibiotic abuse. High resistance against these commonly used antibacterials is certainly worrisome. Therefore these drugs should no longer be prescribed as initial empirical therapy but unfortunately these antibiotics are yet being prescribed as first line drugs in the developing countries.[14] A study done by Laila et al, showed that *E. coli* (46.4%) is the most common pathogen isolated from the urine samples and was resistant to more than one antibiotic. The association of highly urovirulent strains of *E. coli* with antimicrobial resistance mayarise because of prolonged enteric colonization. Exactly how enteric colonization occurs initially and how uropathogenic *E. coli* strains are transmitted among members of the community are not clear.[10] Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control, and information will directly affect selection of empiric therapy for UTI. However, the initial choice of empiric antimicrobial therapy should be based on Gram stain and urine culture and should integrate local sensitivity patterns of the infecting organism. [15]

The absence of antibiotic prescribing policies and inadequate information on patterns of bacterial resistance, may all contribute to the emergence of resistant strains. Therefore, medical practices aimed at avoiding over prescription of antimicrobial agents should be implemented. In addition, strict adherence to hygiene practices is necessary to prevent the spread of resistant organisms.[10]

V. Conclusion

Emergence of multidrug resistant (MDR) *E. coli* has an increasing trend and is a significant clinical challenge, because of limited therapeutic option for this pathogen. Therefore early detection of MDR and Extended spectrum beta lactamase producing *E. coli* is important to restrict their spread in community. Hence, we recommend the continuous monitoring of antibiotic susceptibility in *E. coli* isolates and the resistant isolates to be routinely screened for different kinds of easily detectable virulence factors and beta lactamases to update the characteristics and new types of resistance mechanisms emerging in *E. coli*.

Acknowledgement

We are deeply thankful to the Director and Principal, K.V.G. Medical College & Hospital for their constant encouragement. We are also thankful to all the faculty members and technicians of Department of Microbiology for their help in carrying out this study.

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