VOLUME-6 | ISSUE-4 | APRIL - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Occurrence of virulence markers and profile of antimicrobial susceptibility of uropathogenic E.coli in central India.

Microbiology

KEYWORDS: UPEC, serum resistance, adherence, haemolycin, antimicrobial susceptibility, *Escherichia coli.*

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ABSTRACT

Uropathogenic Escherichia coli, the most frequent pathogens in acute urinary tract infections, take advantage of assortment of virulence properties. Bacterial adherence to and colonization of the urinary tract by UPEC strains are mediated by several types of virulence factors which need to be evaluated.

Total 125 E. coli isolates were screened for different virulence markers viz Serum Resistance (SR), Adherence to epithelial cells (AD), Haemagglutination (HA) and haemolysin production (HL). Their antimicrobial susceptibility was also studied. Out of 125 isolates of E.coli; 35 were from Male, 78 from Female patients and 12 were from Children. SR was commonest as seen in 98 (72%) cases followed by 79 (63.2%) positive for AD while HA and HL production was seen in only 28 (22.4%) and 24 (19.2%) respectively. The virulence markers were present either alone or simultaneously with other virulence markers with varying frequencies. The antimicrobial susceptibility profile showed the strains to be multidrug resistant.

As many as 89.6% UPEC strains display virulence markers. The Serum Resistance and adhesion appears to be the most consistent virulence markers and play a major role in their pathogenicity. Multiple virulence markers may appear simultaneously. These strains are generally multidrug resistant.

INTRODUCTION:-

Urinary tract infection (U. T. I.) is one of the most common infections seen in our country. Many organisms can infect Urinary Tract; but *Escherichia coli* is the most frequent Gram negative bacillus responsible for Urinary Tract Infection which accounts for about 80 to 90% of all Urinary Tract Infections (Maheswari UB, 2013). Urinary tract infections are a major public health concern in developing countries. The *E. coli* that cause urinary tract infection are not all the strains from the intestinal tract but a subgroup selected by factors enhancing extra intestinal survival. Such factors include motility by the aid of flagella, structural features such as fimbriae or pili and chemical adhesion (Emody L, 2003). It indicates that there is a high prevalence of multidrug resistant *E. coli* in UTI (Shafaq Aiyaz Hassan, 2011). *E.coli* is consistently associated with uropathogenicity and is designated as Uropathogenic *E.coli* (UPEC) these isolates express chromosomally encoded virulence markers. These markers of UPEC are expressed with different frequencies in different disease states, ranging from asymptomatic bacteriuria to chronic pyelonephritis. (K Prabhat Ranjan, 2010).

In *E. coli* virulence results from the cumulative impact of one or several special properties or Virulence factors (Vfs), which serve to distinguish potential pathogen from harmless intestinal strains (Stell, 2000). The most important virulence markers associated with UPEC are heamolysin, epithelial cell adhesion, heamagglutination and serum resistance. The most important amongst the virulence markers, are the adhesins that help them to adhere to uroepithelium and serum resistance.

The ability of *E. coli* to cause urinary tract infections is on the increase, while the ease of treating these infections due to multidrug antibiotic resistance to first line antibiotics such as cotrimoxazole, ampicillin and nitrofurantoin is becoming increasingly exclusive. Of greater concern is the recent increase in resistance to fluoroquinolones such as ciprofloxacin and levofloxacin (Karlowsky JA, 2002).

In view of these, the present study was conducted on urinary isolates of *E.coli* to ascertain the frequency of occurrence of four common virulence markers and also to study their profile of antimicrobial resistance.

MATERIAL AND METHOD:-

The present study was conducted for a period of NOV 2015 to NOV 2016. The total 125 isolates of *E. coli* from patients of a clinical diagnosis of urinary tract infection (UTI) and those who had pyuria were included in this study. The strains were stocked on nutrient agar slants at 4^0C and processed within one week of isolation.

.Detection of virulence markers:

1. Hemolycin (HL) production :-

All *E. coli* strains were tested for haemolysin production on 5% Human Blood (O Rh +ve) Agar. The isolates were stabbed on the Blood agar plate and incubated overnight at 37°C aerobically. The yellow clear zone surrounding the colony indicated haemolysis. (Arora, 2010).

2. Heamagglutination (HA) :

Freshly collected Human type O Rh positive erythrocytes were used for the HA test. The erythrocytes were washed three times and suspended to a 3% (v/v) concentration in P.B.S at pH 6.8. Approximately 0.025 ml PBS (one drop) was dropped to on a cool microscopic slide; colonies of *E. coli* were emulsified in PBS to get heavy milky white suspension. An equal volume of erythrocyte suspension was added and gently mixed with a wooden applicator. The slide was gently rotated and macroscopic hemagglutination was observed within 1 min. (Maheswari UB, 2013)

3. Serum resistance (SR) :

Overnight cultures of *E.coli* were grown at 37°C on Muller Hinton agar, harvested and the cells were suspended in 1ml of PBS. Serum (0.05 ml) was added to the suspension. Control tubes contained 0.05 ml of PBS instead of serum. The tubes were then incubated at 37°C for 180 minutes. After incubation the samples were inoculated on the MacConkey agar plates. The plates were incubated for 18 hours at 37°C and the viable count was determined. Susceptibility of bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 180 minutes in relation to the original count of bacteria determined at 0 minutes in the controls. Strains were termed serum sensitive if the viable count dropped to 1% of the initial value and resistant if less than 90% of organisms survived after 180 minutes. (KumarKarnaker, 2014)

4. Adherence to uroepithelial cells (AD):-

The adherence capacity of the different bacterial isolates to uroepithelial cells was assayed as describe by (Svanberg Eden, 1978) . Briefly, squamous and transitional epithelial cells from the urine sediment of one human female donor without a known previous history of urinary tract infection were suspended in PBS. Bacteria (10^8cells) corresponding to 0.5 MacFarland standard were added to 10^5 epithelial cells in PBS with D-mannose diluted to a final concentration of 0.5% in a volume of 1.0 ml. After incubation for 60 min at 37^o C, unattached *E.coli* were eliminated by repeated washing

with PBS and the smear were prepared and stained with Gram stain. The number of *E.coli* attached was observed by directed light microscopy. Adherence is defined as the mean number of bacteria attached to 40 epithelial cells.

Antimicrobial susceptibility:-

Antimicrobial susceptibility testing of all the isolates was done by disc diffusion method by Kirby Bauer 1966 (BaurAW, 1966). Using antibiotic discs of Amoxycillin+Clavulanic acid (AC) (30 mcg), Amikacin (AK) (30 mcg), Nitrofurantoin (NF) (300 mcg), Gentamicin (G) (10 mcg), Ceftazidime (CA) (30 mcg), Ampicillin (A) (10mcg), Ciprofloxacin (CF) (5 mcg), Cefixime (CFX) (5 mcg), Ceftriaxone (CI) (30 mcg), Co-Trimoxazole (CO) (1.25 / 23.75 mcg),Levofloxacin (LE) (5mcg), and Cefuroxime (CU) (30 mcg). (Hi-Media Laboratories).

OBSERVATIONS:-

In the present study out of 125 isolates of *E.coli; 35* were from Male patients, 78 from Female patients and 12 were from Children. Of the virulence markers studied SR was commonest as seen In 90 (72%) cases followed by 79 (63.2%) positive for AD while HA and HL production was seen in only 28 (22.4%) and 24 (19.2%) isolates respectively. Their distribution is shown in Table No. 1. All the virulence markers were present either alone or simultaneously with other virulence markers with varying frequencies and their frequency distribution is shown in Table No. 2. The antimicrobial susceptibility profile showed resistance to almost all the antimicrobial agent tested. Their sensitivity profile is shown in Table No. 3.

Tables:-

Table No. 1: Distribution of Virulence markers in different groups of patients.

Virulence markers	Male (%) N=35	Female (%)N=78	Child (%)N=12	Total (%) N= 125
Heamolycin (HL)	7 (20)	14(17.95)	3(25.00)	24(19.20)
Heamagglutination(HA)	10(28.51)	15(19.23)	3(25.00)	28(22.40)
Adhesion(AD)	19(54.29)	52(66.67)	8(66.67)	79(63.20)
Serum resistance(SR)	24 (68.57)	57(73.08)	9(75.00)	90(72.00)

Table No. 2: Frequency distribution of Occurrence of virulence markers

Virulence markers	Male (%) N=35	Female (%) N=78	Child(%) N=12	Total (%) N= 125
a) Any one				
HL	0(0.00)	1(1.28)	1(8.3)	2(1.6)
HA	1(2.89)	1(1.28)	0(0.00)	2(1.6)
AD	2(5.71)	7(8.97)	2(16.66)	11(8.8)
SR	8(22.85)	15(42.85)	3(25.00)	26(20.8)
Sub Total	11(31.43)	24(68.57)	6(50.00)	41(32.8)
b) Any two				
HL+AD	1(2.89)	0(0.00)	0(0.00)	1(0.8)
HL+SR	3(8.57)	2(2. 56)	0(0.00)	5(4.00)
HA+AD	2(5.71)	3(3.84)	0(0.00)	5(4.00)
HA+SR	0(0.00)	0(0.00)	0(0.00)	0(0.00)
AD+SR	5(14.28)	25(32.05)	3(25.00)	33(26.4)
Sub Total	11(31.43)	30(38.46)	3(25.00)	44(35.2)
c) Any three				
HL+HA+AD	1(2.89)	2(2.56)	0(0.00)	3(2.40)
HL+HA+SR	0(0.00)	0(0.00)	0(0.00)	0(0.00)
HL+AD+SR	2(5.71)	6(7.69)	0(0.00)	8(0.64)
HA+AD+SR	6(17.14)	6(7.69)	1(8.30)	13(10.40)
Sub Total	7(20.00)	14(17.94)	1(8.30)	22(17.6)
d) Any four				
HL+HA+AD+SR	0(0.00)	3(3.84)	2(16.60)	5(4.00)
Total (a+b+c+d)	29(82.85)	71(91.02)	12(100)	112(89.6)

TABLE NO.3: Antimicrobial susceptibility profile of isolates of *E. coli.*

ANTIBODIES	Male (%) N=35	Female (%) N=78	Child (%) N=12	Total (%) N= 125
Amikacin	33(94.29)	73(93.59)	12(100)	118(94.40)
Nitrofurantoin	33(94.29)	73(93.59)	12(100)	118(94.40)
Levofloxacin	33(94.29)	77(98.72)	8(66.67)	118(94.40)
Gentamicin	28(80.00)	66(84.62)	12(100)	106(84.80)
Ceftazidime	29(82.86)	63(80.77)	8(66.67)	100(80.00)
Cefixime	13(37.14)	39(50.00)	4(33.33)	56(44.80)
Cefuroxime	12(34.29)	37(47.44)	6(50)	55(44.00)
Ciprofloxacin	10(28.57)	32(41.03)	12(100)	54(43.20)
Amoxyclav	10(28.57)	34(43.59)	5(41.67)	49(39.20)
Ceftriaxone	12(34.29)	30(38.46)	5(41.67)	47(37.60)
Cotrimoxazole	10(28.57)	24(30.77)	2(16.67)	36(28.80)
Ampicillin	3(8.57)	8(10.26)	0(0)	11(8.80)

DISCUSSION:

Uropathogenic *E.coli* exhibit several virulence markers which confer them with the pathologic potential in the urinary tract. Their frequency varies from place to place (Nataro JP, 1988). In the present study as many as 89.6% strains displayed one or more virulence markers included in the present study. Of the four virulence marker studied, serum resistance (72%) and adhesion (63.2%) were more frequently observed in comparison to heamagglutination (22.4%) and haemolycin production (19.2%) Table no. 1

Nevertheless there were no differences between males, females, and children. The serum resistance appears to be the most consistent virulence markers which probably improve the surviving ability of *E.coli* in human tissues while the next consistent marker was adhesion which enables the bacteria to adhere to the uroepethelial cells.

All four virulence markers under study occurred simultaneously in 4% of all isolates while any three in 17.6%, any two in 35.2% and only one single virulence marker in 32.8%. *E.coli* (Table no. 2) confirming the earlier report of their simultaneous occurrence (Suvara Joshi, 2009)

The antimicrobial susceptibility profile (Table no.3) revealed multi drug resistance in all strains. The *E.coli* isolates showed excellent sensitivity to Amikacin and Nitrofurontoin and good sensitivity with Gentamicin, Ceftazidime, and poor sensitivity with other antimicrobial tested. The pattern was same for males, females, as well as children except Levofloxacin which showed little less sensitive in children (66.67%) and Gentamicin, Ciprofloxacin and Amikacin were sensitive to in all isolates from children. However there were only 12 isolates from children and more isolates from them tested may give different pattern of antimicrobial resistance has been described in various earlier reports. (Abera, 2011) (Seema Mitaal, 2015) (Razieh Dehbanipour, 2016)

Thus the results of the present study confirm the role of various virulence markers in uropathogenic *E.coli*. The four virulence marker detected in the present study, individually or together determine virulence of about 90% of E.coli responsible for causing UTI's. Serum resistance is the most frequently appearing virulence markers followed by adhesion to uroepithelial cells while haemagglutination and haemolycin are relatively infrequent and probably just add to their pathogenioc potential. More than one virulence markers can occur in the uropathogenic *E.coli* and relationship between their virulence and presence need to be further evaluated.

Further, the uropathogenic isolates of *E.coli* are generally multidrug resistance. This may further add to their pathogenicity and can be considered as a surrogate marker of virulence of *E.coli*. The phenotypic methods used in the present study are simple to perform, economical and can be practiced I moderately equipped laboratories with limited resources. The regular study of these parameters certainly helps to understand the disease process in a better perspective and assist greatly for epidemiological studies. This forms the back bone of treatment and preventive policies.

REFERENCES:

1. Abera, M. K.. (2011) Antimicrobial susceptibility patterns of E. coli from clinical

sources in northeast Ethiopia. African Health Sciences,vol 11; 840-845.
2. AC, B. H. (1986) Congo red medium to distinguish between invasive and non-invasive Escherichia coli pathogenic for poultry. Journal of Avian diseases,Jan-Mar 30(1); 117-121.
3. Arora, K. P. (2010) An approach to uropathogenic Escherichia coli in Urinary Tack Infection. Journal of laboratory physicians.2 (2);70-73.
4. Baur AW, K. W. (1966) Antibiotic susceptibility testing by a standard single disk metod. America journal of cliical pathology.45(4); 493-496.
5. Emody L, K. M. (2003) Virulence factors of uropathogenic Escherichia coli. International journal of Antimicrobial agent, 22(2); 529-533.
6. K Prabhat Ranjan, N. R. (2010) An Approach to Uropathogenic Escherichia coli in Urinary Tract Infections. Journal of Laboratory Physicians, 2(2) 72-73.
7. Karlowsky JA, K. L. (2002) Trends in antimicrobial resistance among urinary tract infection isolates of Escherichia. Interational Journal Antimicrob Agents, 46 (8) 2540-2545.
8. Karlowsky, J. A. (2002) Fluoroquinolone –Resistant Urinary Isolates of E. coli from out patients Are Frequently Multidrug Resistant: Results from the North American Urinary Tract Infection Collaborative Alliance – Quinolone Resistance Study. Antimicrob. Agents Chemotherapy.50(6), 2251-2254x.
9. Kumar Karnaker, R. S.(2014) Serum bactericidal resistance in uropathogenic E. coli. International journal of current microbiology and applied sciences, 823-828.
10. M, Hooton. T. (2003) Fluoroquinolones and resistance in the treatment of uncomplicated urinary tract infection. International journal of Antimicrobial agents, 65-72.
11. Maheshwari, U. B., Palavi, S., & Kammili, P. R. (2013)Uropathogenic E.coli in acute Urinary tract infection and urolithiasis. Indian journal of urology Oct-Dec.
12. Maheswari UB, P. S. (2013) Hemagglutination and biofilm formation as virulence markers of uropathogenic Escherichia coli in acute urinary tract infections and urolithiasis. Indian journal of Urology, 277-281.
13. Mittal, S., Sharma, M., & Chaudhary, U. (2014) Study of virulence factors of uropathogenic Escherichia coli and its antibiotic susceptibility pattern. Indian journal of pathology and microbiology, 61-64.
14. Nataro JP, K. J. (1988) Diarrheagenic Escherechia coli. Clinical microbiologyl reviews, 142-201.
15. R, Benge. G. (1988)Bactericidal activity of human serum against strains of E coli by the plasmid genes Tra tof R 100 and is of col U , I K-94. Infect Immunology, 654-659.
16. James R. Johnson (1991) Virulence factors in Escherichia coli urinary tract infection. Clinical Microbiology, Vol 4,80-128.
17. Razieh Dehbanipour, S. R.(2016) High prevalence of multidrug-resistance uropathogenic Escherichia coli strains, Isfahan, Iran. Journal of Natural Science, Biology and Medicine,
18. Seema Mitaal, M. S. (2015) Biofilm and multidrug resistance in uropathogenic Escherichia coli. Pathogens and Global health, 26-29
19. Shafaq Aiyaz Hassan, S. A. (2011) ccurrence of multidrug resistant and esbl producing. Journal of Basic and Applied Sciences, 39-43..
20. Stell, J. J. (2000) Extended Virulence Genotypes ofEscherichia coli Strains from Patients.
21. Suvara Joshi, Thakar Y(2009) Study of virulence marker of Eschershia coli isolated from urinary tract infection. Journal of pure and applied microbiology, 679-683.
22. Svanberg Eden, H. H.(1978)Escherichia coli pilli as possible mediators of attachment to human urinary tract Epethelial cells. Infection and Immunity, 229-237.
23. Tarulata Saikia, J. K. (2016) Detection of Virulence Markers in Uropathogenic Escherichia coli, their Serotypes and Antibiotic Sensitivity Patterns. International Journal of Current Microbiology and Applied Sciences, 784-792.