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Study of Virulence Markers of *E. coli* Isolated from U.T.I.

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A total of 40 urinary isolates of *E.coli* from patients of U.T.I. and 30 faecal isolates from healthy persons were studied for O serotyping; Haemolysin production; Haemagglutination; Congo red binding. Haemolysin was positive in 21 (52.5%) of urinary isolates as against only 9 (30%) of faecal isolates; Haemagglutination was positive in 23 (57.5 %) of which 18(45%) were mannose resistant Haemagglutinating while only 10 (33.3%) of faecal isolates were Haemagglutinating. All the 40 (100%) Urinary isolates and as many as 28(93.3%) out of 30 faecal *E.coli* were able to bind Congo red dye. O serotyping was possible in 65% of uropathogenic and 63.3% faecal *E.coli* strains. The early serogroups viz; O2; O4; O8; O23; O25 were commonest. Urinary isolates of *E.coli* were sensitive most to Nitrofurantoin (97.5%), followed by Amikacin (95%), Gentamicin (82.5%), Ceftazidime (82.5%) and Amoxycillin+Clavulanic acid (75%), while the sensitivity was even less than 50% to Cephotaxime (47.5%), Ceftriaxone (45%), Cefuroxime (37.5%), Cefixime (25%), Ciprofloxacin (22.5%), Cotrimoxazole (17.5 %), Norfloxacin (17.5%), Ampicillin (12.5%). Thus study shows haemolysin and haemagglutination are reliable markers of virulence in uropathogenic *E.coli*. Congo red binding is not good marker and there is prevalence of limited serogroups of uropathogenic *E.coli* in this region and the isolates are generally multidrug resistant.

Key words: Uropathogenic *E. coli*, Urinary tract infections, Haemagglutination, Haemolysin, Congo red binding.

Urinary tract infection is a major bacterial disease among women. Uropathogenic *Escherichia coli* are the most dominant causative agent[1]. Certain O serotypes of *E. coli* have been designated to be consistently associated with U.T.I. The uropathogenic *E. coli* strains possess certain virulence markers that are expressed in different frequencies. Many studies have been carried out in patients of U.T.I to assist the presence of various virulence markers[2,3,4,5,6] where as data from commensal faecal flora is rather fragmentary. In the present study an attempt is made to ascertain common virulence markers viz; Haemolysin production; Haemagglutination production; Congo red binding and O Serogrouping in *E. coli* isolated from patients of U.T.I as well as their occurrence in the commensal gut isolates.

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MATERIAL AND METHODS

The study comprises of 40 isolates of *E. coli* obtained from Urine of patients of U. T. I. In addition 30 *E. coli* isolates obtained from stool specimens of healthy individuals without history of U. T. I. or diarrhea within last six months were included as controls.

Antimicrobial susceptibility testing of all the isolates was done by disc diffusion method[7]. using antibiotic discs of Amoxycillin+Clavulanic acid (AC) (30 mcg), Amikacin (AK) (30 mcg), Nitrofurantoin(NF) (300 mcg), Gentamicin (G) (10 mcg), Ofloxacin (OF) (5 mcg), Ceftazidime (CA) (30 mcg), Ampicillin (A) (10mcg), Cephotaxime (CE) (30 mcg), Ciprofloxacin (CF) (5 mcg), Cefixime (CFX) (5 mcg), Ceftriaxone (CI) (30 mcg), Co-Trimoxazole (CO) (1.25 / 23.75 mcg), Norfloxacin (NX) (10 mcg), Cefuroxime (CU) (30 mcg), Cefoperazone (CS) (75 mcg). (Hi-Media Laboratories)

Hemolysin Production

All *E. coli* strains were tested for haemolysin production on 5% Human Blood (O Rh +ve) Agar[4]. The isolates were stabbed on the Blood agar plate and incubated overnight at 37ºC aerobically. The yellow clear zone surrounding the colony indicated haemolysis.

Haemagglutination test

It was done by method described by Evans *et al* (1979)[8]. Human type O Rh positive erythrocytes freshly collected 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. The bacteria were grown on CFA agar . Approximately 0.025 ml PBS (one drop) was dropped on to cool microscopic slide; colonies of bacteria were emulsified in PBS to get a 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 Haemagglutination was observed within 1 min.

Haemagglutination inhibition test with D- mannose: The ability of D – mannose to inhibit HA was tested at a concentration of 25 mg/ml of PBS at pH 6.8 as described by Hagberg *et al* (1981)[9]. A drop of D –mannose suspension was added to the slide showing macroscopic HA. The slide was gently rotated for one minute and read for HA type. HA was typed as mannose resistant Haemagglutination (MRHA) if the same degree of HA occurred with or without mannose and mannose sensitive Haemagglutination if HA was prevented or grossly reduced in the presence of D – mannose.

Congo red binding

Congo red binding was assayed as described by Berkhoff and Vinal (1985)[10]. Briefly, strains were grown in LB medium (37^{0}C, 24 hrs.) and seeded onto CR agar (Tripticase Soya Agar supplemented with 0.03% Congo red dye and 0.15% bile salts). The cultures were incubated for 24 hrs (37^{0}c). Congo Red positive *E. coli* isolates were identified by the appearance of red colonies.

O serotyping

O serotyping of the isolates was done at national Salmonella and Escherichia Centre; Central Research Institute; Kasauli.

RESULTS AND DISCUSSION

Out of 40 urinary isolates of *E. coli* 21 (52.5%) were Haemolysin positive as against only 9 (30%) of the 30 faecal isolates of *E. coli*.

Various studies have also reported increased percentage of Haemolysin production in Uropathogenic *E. coli*. However the incidence varies widely[2,3,5,11,12]. Generally, *E. coli* isolated from upper Urinary Tract Infection have been reported to have high incidence of Haemolysin production. As compare to isolates obtained from lower urinary tract[4]. This discrimination of upper and lower Urinary Tract Infection is not considered in many studies including the present

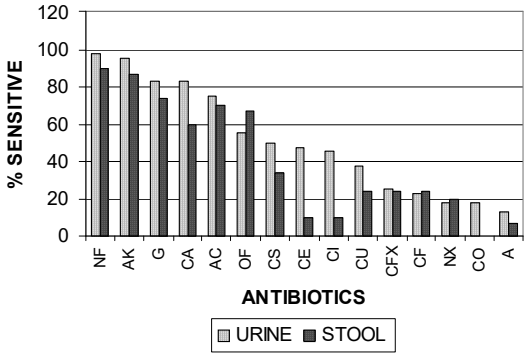

Fig. 1. Antibiotic susceptibility profile of the isolates of *E. coli*

one and could have resulted in variation in the incidence of Haemolysin production.

The Haemagglutination was positive in 23 (57.5%) of Urinary isolates *E. coli* and out of this 18 (45%) were mannose resistant Haemagglutinating of *E. coli* while only 10 (33.3%) fecal isolates of *E. coli* showed Haemagglutination of which 7 (23.3%) were Mannose Resistant haemagglutinating.

The incidence of mannose resistant Haemagglutination varies widely in different studies[2,5,11]. MRHA *E. coli* are known to be associated with severe forms of Urinary Tract Infection[9]. The MRHA strains have been found to have a predilection to adhere more to upper Urinary Tract and than to the lower urinary tract[5,9]. Hence, it can be considered a very simple and useful laboratory marker to evaluate the severity of the infection.

All the 40 (100%) urinary isolates and as many as 28 (93.3%) out of 30 fecal *E. coli* were able to bind Congo red dye. Congo red binding has been found to be a useful virulence marker in avian septicemic strains[10]. This virulence marker has also reported to be associated with pathogenicity of *Shigella flexneri*[13], *Yersinia pestis*[14], *Vibrio cholerae*[15].

Table 1. Correlation between Haemolysin Production and Haemagglutination Production

Test	Urine specimen	Stool specimen
Only HL positive	08 (20%)	7 (23.3%)
Only HA positive	10 (25%)	6 (20 %)
Both HL and HA positive	13 (32.5%)	4 (13.3%)
Sub total	31 (77.5%)	17 (56 %)
Both HL and HA negative	9 (22.5%)	13 (43.3%)
Total	40 (100%)	30 (100%)

Table 2. 'O' serotyping of *E. coli* isolates

O serotypes	Urine (40)	Stool (30)
1	0	3
2	1	6
4	5	0
8	7	2
23	1	0
25	3	1
53	1	0
60	1	1
77	0	1
78	0	1
102	1	0
109	1	0
116	0	2
130	0	1
138	3	0
147	1	0
148	1	0
167	0	1
Rough	1	2
UT	13	9
Total	40	30

The First study conducted by Silveria *et al* (2001)[6] has reported a good absorption of Congo red dye by uropathogenic *E. coli*.

In the present study also all the Urinary isolates were positive for Congo red binding. However, high proportions i.e. 93.3% of faecal isolates were also positive for Congo red binding. Thus the findings suggest that Congo red binding test is unable to discriminate between uropathogenic and non-uropathogenic *E. coli*.

The results in the present study point out that the ability of *E. coli* to bind Congo red dye is more consistent for human isolates than being the virulence marker.

Correlation between Haemolysin Production and Haemagglutination Production: Correlation between Haemolysin Production and Haemagglutination Production is shown in Table 1. In the present study, the haemolysin production and haemagglutination were singly or together present in 77.5% uropathogenic *E. coli* as against only 56% in faecal isolates. In the

etiopathogenesis of U.T.I. it is considered that faecal *E. coli* can cause infections in the urinary tract. The strains responsible for this are more likely to be these virulence factor producing strains than the other ones present as commensals in faeces. The correlation between Haemagglutination and Haemolysin production was simultaneously accounted for 32.5% strains.

Few studies have also reported that there is no coexistence between Haemolysin and Haemagglutination production in Uropathogenic *E.coli*[2,3]. Strains with both Hemolytic and Haemagglutination properties have been related with high incidence of pyelonephritis[16].

O Serogrouping profile of *E.coli* isolates is shown in table 2. In the present study, 65% of Uropathogenic & 63.3% faecal *E. coli* strains were typable. The typability of Uropathogenic *E. coli* has reported to be ranging from 13.7% to 76.8%[17].

Wide variation in the typability of *E. coli* strains is obtained due to use of limited sets of O-antisera. Apart from this geographical distribution also determines the incidence of the locally prevalent groups against which the antisera may not be available[17].

A low incidence of rough strains of *E. coli* was observed in our study. These have been reported to emerge due to degenerative changes that the organism undergoes due to constant exposure to O-antibodies.

In the present study, the early serogroups namely O2, O4, O8, O23 and O25 were comments and comprised of 42.5% isolates.

In Indian situation diverse groups of verity of O serogroup have been encountered. Arora and Chitkara (1972)[18], found O2, O4, O6 and O18 to be more prevalent, while Prabhu *et al* (1981)[19] reported 76% of typable isolates belonged to the first 10 serogroups, the common sero groups being O6, O4, O2, and O1.

In contrast Pande *et al* (1974)[20] found O18, O20, O44, O56, O86 and O112 to be common. Fule *et al* (1990)[2] found O57, O20 and O90 to be common while Gupta *et al* (1991)[17] reported O90, O21 and O68 to be more frequently encountered.

Antimicrobial susceptibility profile is depicted in Fig. 1. In the present study, the antibiotic sensitivity of urinary tract isolates of *E. coli* was found to be most to Nitrofurantoin (97.5%), followed by Amikacin (95%), Gentamicin (82.5%), Ceftazidime (82.5%) and Amoxycillin and Clavulinic acid (75%), while the sensitivity was even less than 50% to Cephotaxime (47.5%), Ceftriaxone (45%), Cefuroxime (37.5%), Cefixime (25%), Ciprofloxacin (22.5%), Cotrimoxazole (17.5%), Norfloxacin (17.5%), Ampicillin (12.5%). The antibiotic sensitivity of profile of stool also revealed more or less similar sensitivity pattern.

This indicates that multidrug resistance *E. coli* strains are prevalent in the region. It is interesting to observe that resistance was more frequently observe with oral antibiotic like Ofloxacin, Ampicillin, Ciprofloxacin, Cefixime, Cotrimoxazole, Norfloxacin and Cefuroxime which are usually given empirically to the patients for the treatment to Urinary Tract infection.

Multidrug resistant strains have also been reported by other studies[2,3]. Multidrug resistance in clinical isolates may also be considered to be one of the virulence factors. Thus in all the cases of Urinary tract infections it is essential to isolate the pathogen in culture add perform antimicrobial susceptibility testing to advocate the rational antibiotic therapy.

The findings in the present study show that both haemolysin and Haemagglutination are reliable markers of virulence in Uropathogenic *E. coli*. The Congo red binding can not be used as a marker for Uropathogenic *E. coli* as it does not discriminate well between Urinary and Faecal isolates. There is prevalence of limited serogroups of Uropathogenic *E. coli* in this region and the isolates are generally multidrug resistant.

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REFERENCES

1. Steadman, R., Topley, N. The virulence of Escherichia coli in urinary tract. In: *Urinary tract infections*. (Brumfitt W, Jeremy MT, Hamilton Miller. Eds) 1st Ed. London: Chapman and Hall publication, 1998; 37-41.

2. Fule, R. P., Menon, S., Saoji, A. M. Antibiotic resistance, haemagglutination type and haemolysin production in relation to serogroups of uropathogenic *E. coli*. *Indian J. Med. Res.*, 1990; **91**: 270.
3. Kaur, P., Jayasheela, M., Chakraborty A. and Saxena, S. N., Kasauli. Characteristics of E. coli isolates from cases of significant bacteriuria. *Indian J. Med. Microbiol*., 1991; **9**: 107.
4. Green, C. P. and Thomas, V. L. Haemagglutination with human O type erythrocytes, hemolysin production and serogrouping of *E. coli* isolated from patient with acute pyelonephritis, cystitis and Asymptomatic bacteriuria. *Infection and Immunity.* , 1981; **31**(1): 309.
5. Raksha, R and Srinivasa H. Occurrence and Characterization of Uropathogenic E. coli in U. T. I. *Indian J. Med. Microbiol*., 2003; **21**(2): 102-107.
6. Silveira, W. D., Benetti, F., Lancellotti, M., Ferrira, A., Sollerini, V. N., and Brocchi, M. Biological and genetic characteristics of Uropathogenic E. coli strains. *Rev. Inst. Med. Trop. S. Paulo*., 2001; **43**(6): 303-310.
7. Bauer, Kirby, Sherris and Turck., Antibiotic sensitivity method. *Am. J. Clin Path.*, 1966; **45**: 493.
8. Evans,D.J., Jr. Evans, D. G.,Dupont, H. Haemagglutination patterns of Enterotoxigenic and Enteropathogenic *E.coli* determined with human, bovine, Chicken and Guinea pig erythrocytes in the presence and absence of mannose. *Infection and Immunity.*, 1979; **23**: 336.
9. Hagberg, L., Jodal, U., Lidin. Janson, G., Lindberg, V., Svanberg Eden C. Adhesion, Haemagglutination and Virulence of E. coli causing UTI's. *Infection and Immunity.*, 1981; **31**: 564.
10. Berhoff, H. A. and Vinal, A. C. Congo red medium to distinguish between invasive and non invasive E. coli pathogenic for poultry. *Avian. Dis.*, 1985; **30**: 117-121.
11. Blanco, J., Alinoso, M.P., Balanco, M., Blanco, J.E., Gonzales E. A., Garbal, J. I. Establishment of three categories of P fimbriated E. Coli Stains that show different toxic phenotypes and belong to particular O serogroups. *F. E. M.S. microbiol. Lett.* Dec. 7, 1992 ; **78**: 131.
12. Bhalla, P., and Agrawal, P. Urinary E. coli prevalent serotypes, antimicrobial susceptibility and virulence factors. *Indian J Med Microbiol.*, 1989; **7**: 188.
13. Maurelli, A. T., Blackmon, B. and Curtiss III, R. Loss of pigmentation in Shigella flexneri 2a is correlated with loss of virulence & virulence associated plasmid. *Infect. Immun.*, 1984; **43**: 397-401.
14. Surgalla, M. J. & Beesley, E. D. Congo red agar medium for detecting pigmentation in Pasteurella pestis. *Appl. Microbial.*,1969; **18**: 834-837.
15. Payne, S. M. and Finkelstein, R. A. Detection and differentiation of iron responsive avirulent mutants on Congo red agar. *Infect. Immun*., 1977; **18**: 94-98.
16. Hellana, L., Lars, A. H. , Do. J. Vlt Jodal Lefllar H. and Svanberg Eden. Correlation of P bld group, Vesicourerteral reflex and bacterial attachment in patients with recurrent pyelonephritis. *New Eng. J. of Med.*, 1983; **309**: 1189.
17. Gupta, S., Kaur, P. and Agrawal, P. Prevalence of *E. coli* 'O' serogroup in India (1985 - 87). *Indian J. Med. Microbiol*., 1991; **9**(3): 142.
18. Arora, S and Chitkara, N. L. E. coli serotypes prevalent in Urinary Tract Infections at Amritsar. *Indian J. Path Bact.*, 1972; 15 : 62.
19. Prabhu, A., Mondekar, A. D., Nadkarni, M. S. Hemolysin and necrotoxin production of E. coli with special reference to serotypes causing Urinary Tract Infection. *Indian J. Path Microbiol*., 1981; **24**: 33.
20. Pande, R. C., Gupta, S. C., Rajvanshi, V. S. and Mehrotra, T. N. Hemolysin and necrotoxin production by E. coli *Indian J. Med. Res*., 1974; **62**: 1448.