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DETECTION AND PREVALENCE OF VIRULENCE MARKERS OF *E.COLI* ISOLATED IN AND AROUND ERODE

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ABSTRACT
Urinary tract infections (UTIs) are significant health problem, with *Escherichia coli* (*E. coli*) as the primary pathogen in proximally 80% of the cases. Many virulence factors like Cell surface hydrophobicity (CSH), plasmid, Hemagglutinin, Siderophore, ESBLs *etc.* are responsible for the development of infections. Results showed the presence of these virulence factors in the isolated *E. coli* strains. Siderophore and CSH found to be more prevalent in *E. coli* and their isolation rate was found to be 32.17% and 31.30% respectively. Most of the *E. coli* strains were isolated from female patients.

KEY WORDS: Urinary tract infections, Cell surface hydrophobicity, plasmid, Hemagglutinin, Siderophore, ESBLs.

INTRODUCTION

Urinary tract infections (UTIs) remain a common clinical problem in both the community and health care associated settings. (Tony mazzulli, 2012) Each patient should be carefully assessed, diagnosed and an appropriate antimicrobial therapy must be instituted. This will contain the growing tide of antimicrobial resistance and allow for the continued use of simpler, less expensive antimicrobial agents. *E. coli* is the most frequent urinary pathogen isolated from 50–90% of all uncomplicated urinary tract infections. The present study was designed to detect 6 virulent markers of *E. coli* isolated from the patients of UTI and their prevalence rate in the area of Erode. Since no had carried out such studies in this area, we had undertaken this task to find out them and comparing them with the world wide data. An attempt has been made to validate the relative importance of six proposed virulence factors namely hemolysin, hemagglutination, cell surface hydrophobicity, siderophore and plasmid. A study of this category can definitely help the physicians or scientists to find out a right antimicrobial agent or may take up a methodical approach for a formulation of a vaccine against UTI in the future.

MATERIALS & METHODS

This study was conducted in the Department of Microbiology, K.S.R Institute of Dental Sciences and Research, Tiruchengodu from June 2011- April 2015. Two Hundred and thirty *E. coli* strains were isolated from both Symptomatic and Asymptomatic patients. Urine samples were processed and *Escherichia coli* were isolated and identified according to the standard protocols. (Mackie & McCartney, 1989) The isolates were maintained by inoculating them in the nutrient agar butts and stored at room temperature and tested for virulent markers.

Detection of virulence factors

Hemolysin production

The plate hemolysis test was done for the detection of α-hemolysis produced by the *E. coli*. The bacteria were inoculated into 5% sheep blood agar and incubated overnight at 35°C. Hemolysin production was detected by the presence of a zone of complete lysis of the erythrocytes around the colony and clearing of the medium (*Bhat et al.*, 2007).

Siderophore production assay

This test was carried out by using a method named 'chrome azurol sulphonate (CAS) agar diffusion assay. The chrome aurol sulphonate (CAS) assay detects colour change of CAS-Iron complex from blue to orange after chelation of the bound iron by siderophores. A strong ligand 'L' (*e.g.*, a siderophore) is added to a highly coloured iron dye complex; when the iron ligand complex is formed, the release of the free dye is accompanied by a colour change (Vagarali, 2008).

Salt Aggregation Test for detection of Cell Surface Hydrophobicity (CSH)

Bacteria were tested for their hydrophobic property by using different molar concentrations of ammonium sulphate. Those which aggregated with salt particles and formed clumps were considered hydrophobic (*Bhat et al.*, 2007).

Hemagglutination

The Hemagglutinins were detected by agglutination of erythrocytes from human blood group O (Mackie & McCartney 1989).

Detection of Extended spectrum β- lactamases

All the isolates were screened for ESBL production by using three indicator third generation cephalosporins,

ceftazidime (30 µg), cefotaxime (30 µg) andcefpodoxime (30µg). Thezone diameter of < 22 mm for ceftazidime, < 27 mm for cefotaxime and < 17 mm for cefpodoxime was recorded as resistant (*Bhat et al.,* 2007).

Plasmid isolaton

This technique was carried out by alkaline lysis method

RESULTS

Two thousand four hundred and eighty three specimens were collected. Out of 2483, 1608 were collected from symptomatic patients and the remaining (875) were collected from asymptomatic patients. A total of Two hundred and thirty *E. coli* strains were isolated and its isolation rate was found to be 9.262%. Out of 230 *E. coli* strains, 202 and 28 *E. coli* strains were isolated from Symptomatic patients and asymptomatic patients respectively. 84.347% of *E. coli* strains were isolated from Women and the remaining was isolated from men. This was in very much in concordance with world data and other workers (Shaon ray chaudhuri *et al*., 2008) Results were tabulated in the table no .1

TABLE 1: Isolation rate of *E. coli*

S.No	No of Urine specimen	No. of *E.coli* isolated	Percentage of isolation
1.	2483	230	9.262

The major virulent markers seen in our study were Siderophore, followed by Cell surface hyrophobicity, Hemolysin production. Hemagglutination, Plasmid and ESBL production. Results were tabulated in Table 2.

TABLE 2: Screening and rate of detection of Virulence factors

S.No	Name of the virulent factor	No of *E. coli* strains manifesting the virulent factor (n=230)	Percentage of *E. coli* strains manifesting the virulent factor
1.	Siderophore production	74	32.173
2.	Cell surface hydrophobicity	72	31.304
3.	Hemolysin production	71	30.869
4.	Hemagglutination	60	26.086
5.	Plasmid	55	23.913
6.	ESBL	26	11.304

TABLE 3: Strains carrying Number of virulence factors

S.No	No of Virulence factors	No of strains carrying Virulence factors (n = 230)
1	1	110
2	2	37
3	3	17
4	4	23
5	5	5
6	6	2
7	Zero	36

E. coli strains were more isolated from symptomatic patients than asymptomatic patients and the details of isolation of strains were given in the table no.4

TABLE 5: The percentage of isolation was found to be more in Symptomatic than in asymptomatic group

S. No	Group	No of specimen collected	No of E.coli strains isolated	Percentage of isolation
1.	Symptomatic	1608	202	12.56
2.	Asymptomatic	875	28	3.2

DISCUSSION

Women were found to be more infected than males, the reasons ascribed were the short urethra and the anogenital distance in females is small. Even promiscuous activities can escalate UTI in females (Bailey & Scott 9th Edition). The occurrence of multiple virulence factors in the UPEC strains further strengthens the concept of the association of UPEC with urinary pathogenicity. Strains with virulence factors had been tabulated in the table no 3.

In the present study 36.52 % of E.coli strains were found to be carrying multiple virulence factors (more than 1) where as 47.82% percent of them carrying only one virulence factor. Thirty six strains (15.7%) were found to be carrying Zero virulence factor establishing the fact that virulence factors were indeed needed for pathogenicity for most of the strains. There is a chance that E. coli strains which were found with no virulence factor, might have some other virulence factor which we had not checked. It is possible that higher mortality and severity of infection caused by virulence factor producing isolates is due to the expression of several virulence genes simultaneously. In our study strains with siderophore productions were more isolated which was very much in concordance with vagarali 2007. Other workers reported Hemolysin production was higher (Gholamhoseinina *et al*., 2006) and Jwan ahmed ali 2012. Virulence factor production reports

from workers seem to be different in the different areas. An Epidemiological study of this virulence factor production will definitely help us to the have the picture of their expression, which can also help us to formulate a primitive vaccine.

CONCLUSION

As UTI was found to be one of the top most infection in the world , So an epidemiological study of this sort will be very helpful to clinicians of today and future , and this can also lead to the containment of infection by giving the right proscription and prescriptions to the society at the large.

RECOMMENDATION

More number of E.coli strains with virulence factors must be studied in conjunction with their antibiogram. So we can get a complete picture of the real scenario of E.coli causing UTI in this world

REFERENCES

Bailey & Scott's Diagnostic Microbiology, 9th Edition. Mosby publication, 1994.

Bhat, G.K., Sharma, S., Shenoy, S. (2007) Virulence factors and drug resistance in *E.coli* isolated from extra intestinal infections, IJMM; 25 (4): 369-73

Gholamhoseinian Najar, A., Mosavi Nejad, M. and Manouri, S. (2006) The comparison between virulence factors of *Escherichia coli* isolated from urinary tract infections and feacal flora. Research in pharmaceutical sciences, 2:99- 103.

Jwan Ahmed Ali (2012) Hemolysin and Bacteriocin production of *E.coli* isolated from Urinary tract infection. Journal of Babylon University/pure and Applied sciences / / vol.20 (5):1448-1451.

Mackie & McCartney, Practical Medical Microbiology, 13th Edition Vol.2, Churchill Livingstone publication 1989.

Shaon Ray Chaudhrui, Ashoke Ranjanhkur, Poulome Nandy, Santanu Samanta. Urinary tract infection – A survey of local population. American journal of infectious diseases 4 (2): 117- 223, 2008

Sharma, S., Bhat, G. K., Shenoy, S. (2007) Virulence factors and drug resistance in *E.coli* isolated from extra intestinal infections. IJMM 25 (4):369-73

Siegfried, L., Marta Kmetova, Hana Puzove, Maria Molokacova and Filka, J. (1994) Virulence- associated factors in *Escherichia coli* strains isolated from children with urinary tract infections, *J. med Microbiol*; **41**: 127-132.

Tony mazzuli (2012) Diagnosis and management of simple and complicated urinary tract infections (UTIs). The Canadian journal of urology; 19 (supplement1) October 2012.

Vagarali, M.A., Karadesai, S.G., Patil, C.S., Metfud, S.C., Mutnal, M.B. (2008) Haemagglutination and siderophore production as the urovirulence marker of uropathogenic *Escherichia coli*. IJMM, 26:68-70