



VIRULENCE FACTORS OF *E. COLI* AND THEIR ASSOCIATION WITH DRUG RESISTANCE – A PRIMITIVE STUDY

^aSarath Chandra Kandekar, S. & ^bChandra Sekaran, S.

^aDept. of Microbiology, K.S.R Institute of Dental Sciences and Research, Tiruchengodu, Tamil Nadu, INDIA – 637 215

^bDept. of Microbiology, VMKV Medical College, Salem -636 308

ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the vast majority of urinary tract infections (UTIs) including cystitis and pyelonephritis. (Justyna bien, 2012) Strains with multiple virulence factors and multi drug resistance were isolated. 92% of the strains were found to be resistant at least one antibiotic, suggesting the alarming situation in the community. These resistant strains were found to possess the multiple virulence factors. Most of the resistant strains were found to possess either Siderophore producing or hydrophobic or showing hemagglutination. All the ESBL strains were resistant to few antibiotics.

KEY WORDS: *Escherichia coli*, Siderophore, urinary tract infections, Hemagglutinin.

INTRODUCTION

Escherichia coli (*E. coli*) is one of the most important causes of Urinary tract infections (UTIs), including cystitis and pyelonephritis which are the most common infections in childhood. *E. coli* accounts for as much as 80% of UTIs. The pathogenic potential of *E. coli* is thought to be dependent on the presence of virulence factors (VFs). (Johnson,1991). The present study was undertaken because of lack of more data regarding the association of virulence factors with drug resistance. This study can be helpful in the future regarding the customizing the treatment or in formulating the vaccine.

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 - 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 .G.K 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 auroil 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 - lactamases:

All the isolates were screened for ESBL production by using three indicator third generation cephalosporins, ceftazidime (30µg), cefotaxime (30µg) and cefpodoxime (30µg). The zone diameter of <22 mm for ceftazidime, < 27mm for cefotaxime and <17 mm for cefpodoxime was recorded as resistant (Bhat .G.K et al., 2007).

Plasmid isolation

This technique was carried out by alkaline lysis method (Birnboim ,1979). Antibiogram was performed according to Kirby- Bauer disc -diffusion method. The antibiotic discs were procured from Hi Media, Mumbai.

RESULTS

Two hundred and thirty *E. coli* strains were isolated from the urine specimen collected from both Symptomatic and

Asymptomatic groups. Most of the *E. coli* was isolated from Symptomatic group. Results were tabulated in the Table 1.

TABLE 1: Details of Specimen collection.

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

Most of the *E. coli* strains were found to be resistant to gentamycin and other commonly used drugs. Results of the resistant strains were tabulated in the table 2.

TABLE 2: Results of resistant strains

S.No	Name of the antibiotic	No.of resistant strains (n=230)	Percentage of resistant strains
1	Gentamycin	142	61.73
2	Norflox	53	23.04
3	Amikacin	50	21.73
4	Oflaxacin	47	20.43
5	Ampicillin	45	19.56
6	Ciprofloxacin	41	17.82
7	Amoxycillin	39	16.95
8	Lincomycin	29	12.60
9	Norflox	20	8.69
10	Cefodroxil	12	5.21
11	Roxithromycin	12	5.21
12	Cefotaxime	4	1.73
13	Cephalexin	3	1.3
14	Azithromycin	3	1.3
15	Cefopime	3	1.3
16	Piperacillin / Tazobactam	2	0.89

Presence of 6 virulent factors had been checked in all the isolates. Siderophore production was found in 32.17% of the strains. Remaining results had been tabulated in the table 3.

TABLE 3: *E. coli* strains showing Virulence factors.

S.No	Name of the virulence factor	No of strains showing virulence factor (n=230)	Percentage of strains showing virulence factor
1	Siderophore production	74	32.17
2	Cell surface hydrophobicity	72	31.3
3	Hemolysin production	71	30.86
4	Hemagglutination	60	26.08
5	Plasmid	55	23.91
6	ESBL	26	11.3

Most of the *E. coli* strains were found to be harboring a minimum of one virulence factor. Some were found to be harboring all the 6 virulence factors. Results were tabulated in the following figure 1.

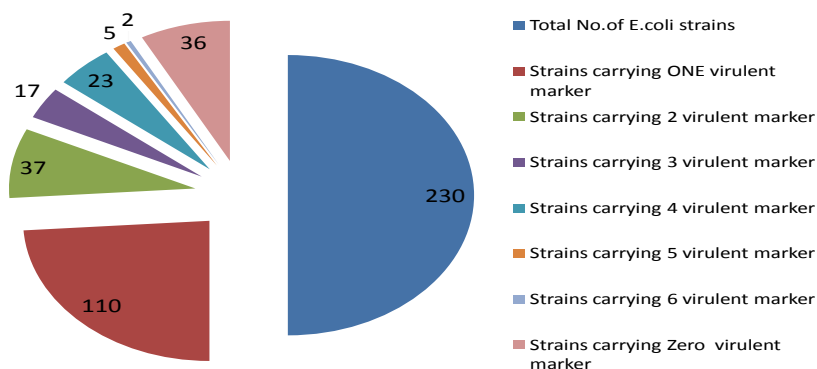


FIGURE 1: Details of the strains showing the production of different number of virulence markers.

Most of the Siderophore producing *E. coli* strains were also found to be harboring other virulent markers but the vice versa was not much seen. Results of the siderophore producing *E. coli* strains were given in the figure 2.

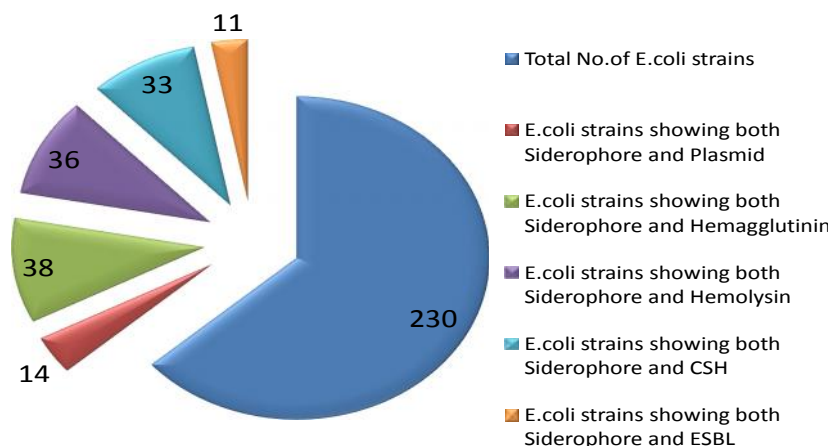


FIGURE 2: Profile of siderophore carrying *E. coli* strains.

DISCUSSION

Most of the siderophore producing strains (92%) were found to be resistant to at least one antibiotic and we also found that most of the strains carrying virulence factor had shown the resistant feature to at least one of the antibiotic tested. Most of the hemagglutinating strains were found to be hemolytic, hydrophobic and producing siderophore. In our study siderophore producing strains were found to be more (32.17%) and the number of strains (31.30%) showing Hydrophobicity were found to be equal to siderophore producing strains, where as Bhat GK 2007 reported hydrophobic strains were more isolated in his study which was concordant with ours. The present study also showed that an average of 30% of the strains produced hydrophobicity, siderophore and hemolysin. Most of the virulence factor containing strains was found to be resistant to at least one antibiotic. Even though we cannot ascertain the relation between the two, at the same time the possibility of relation cannot be neglected. Even though the virulence of an organism cannot be precisely predicted on the basis of its expressive phenotypic virulence factor, presence of multiple virulence factors does increase the virulence of organisms (Bhat, 2007). A total of 99 strains (43.04%) were found to be resistant at least to 2 or more than 2 antibiotics suggesting the drug resistance in the community is alarming. On an average more than 90% of strains found to possess the drug resistant characteristics and a virulence factor. It suggests there may be a relation between the resistant factors and virulence factors.

All ESBL producers were found to be resistant to one or more antibiotics tested and some of the ESBL producers were not plasmid mediated. The greater prevalence of resistance to common antibiotics was reported by other workers Bhat 2007, Chitnis 2003 and Wiener 1999. We do find the same in our study.

The present study had shown the ability of *E. coli* to adapt and survive in different tissues by developing drug resistance and by producing different virulence factors. It

was very clear that the drug resistance was alarmingly soaring. If we could find the relation between the virulence factors and the drug resistance, probably it may help us to prescribe the right antibiotic and may help us to make a vaccine by taking all the finding into consideration.

CONCLUSION

Multi drug resistant strains with multiple virulence factors had been isolated in our study. Judicious use of antibiotics and good prescription policies should be instituted to contain the dissemination of drug resistant strains within the community.

RECOMMENDATIONS

We recommend checking the other Epidemiological parameters of UTIs. Studies of such sort would definitely help the community as well as the whole world.

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