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International Journal of Current Research
Vol. 5, Issue, 09, pp.2613-2616, September, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

THE STUDY OF VIRULENCE FACTOR PRODUCTION IN ESCHERICHIA COLI CAUSING URINARY TRACT INFECTIONS

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ARTICLE INFO

Article History:
Received 05th June, 2013
Received in revised form
16th July, 2013
Accepted 29th August, 2013
Published online 14th September, 2013

Key words:
Virulence factors,
E.coli,
UTI.

ABSTRACT

Urinary tract infection is one of the most common cause of bacterial infections and *Escherichia coli* is the predominant urinary pathogen isolated from community based and hospitalized patients.[1,2] Uropathogenic strains of *E.coli* are consistently associated with UTI exhibiting various virulence factors like serum resistance, cell surface hydrophobicity and hemolysin.[2] The treatment has been posing as a challenge due to the emergence of multidrug resistant *E .coli* especially in nosocomial infections. The present study is undertaken to evaluate association of virulence factors of *E. coli* in comparison to antimicrobial resistance. Total of 100 *E.coli* isolates from clinically suspected UTI cases from patients attending Victoria hospital & Vani Vilas hospital attached to BMC & RI, Bangalore were studied from October 2010 to September 2012. Mid stream urine samples were collected after taking informed consent from all patients and were processed. All samples were cultured by semi quantitative method. The identification of *E.coli* was done using standard biochemical tests. *E.coli* thus identified were screened for Virulence factors like Serum resistance, Cell surface hydrophobicity and α hemolysin production and studied for the drug resistance patterns. Out of the 100 *E.coli* isolated from UTI cases 45 isolates were from male and 55 from female. Amongst these isolates 48.3% showed Serum resistance, 39.3% showed Haemolysin production and 24.7% showed cell surface hydrophobicity. The antibiotic sensitivity testing showed maximum sensitivity to Amikacin and least to Ampicillin with 87% and 10% respectively.71% of the isolates were resistant to Cefotaxime. Considering high morbidity in UTI the occurrence of virulence markers in Uropathogenic *E.coli* (UPEC) strains strengthens the association with pathogenecity. Hence the screening of above mentioned virulence factors can be routinely done in clinical laboratory.

INTRODUCTION

Urinary tract is the most common site of bacterial infection both in community and hospitalized patients. UTI is an important cause of morbidity and mortality with *E. coli* being the most common pathogen, accounting for 85% of community acquired infections .[1] It is the most frequent urinary pathogen isolated from 50-90% of all uncomplicated UTI.[4] Some strains of *E.coli* can diverge from their commensal cohorts taking on a more pathogenic nature and the ability to cause disease both within the intestinal tract and elsewhere in the host. These pathogenic strains are broadly categorised as diarrhoegenic *E.coli* or extra intestinal pathogenic *E.coli* (*ExPEC*).[5] ExPEC have the capacity to disseminate and colonise other host niches including blood, CNS and urinary tract resulting in diseases.[5]Among ExPEC certain strains were consistently associated with uropathogenecity and were designated as Uropathogenic *E.coli* (UPEC). These isolates express chromosomally encoded virulence markers.[6] These markers of UPEC are expressed with different frequencies in different disease states ranging from asymptomatic bacteruria to Pyelonephritis and also in different patient population.[6] UPEC strains possess specialized virulence factors, enabling them to colonize and invade to the host, disrupt the host defence mechanisms, injure host tissues, and/or stimulate a noxious host inflammatory response thus enhancing their pathogenecity.[7] The term "virulence" (the Latin word meaning poisonous) is defined as the quantitative ability of an organism to cause disease in a particular host.[9,10] The recognized virulence factors of *E.coli* to cause urinary tract infections include increased adherence to uroepithelial cells, resistance to serum bactericidal activity, a higher quality of K antigen (K1, K5,) in capsules, the presence of aerobactin, cytotoxic necrotising factor type 1, haemagglutination, cell surface hydrophobicity, siderophores, hemolysin and gelatinase production.[11,12] UTI is usually treated empirically without culture but it contributes to 10-15% prolongation of hospital stay due to its ability to exhibit resistant bacteria in hospital.[3] In the past, most *E. coli* isolates were highly susceptible to a broad range of antimicrobial agents including beta lactams accounting for 60% of antibiotic use. The prevalence of resistance to first generation cephalosporin and trimethoprim-sulfamethoxazole is increased to about 23.4% and 33.7% respectively. Resistance of *E. coli* to fluoroquinolones has increased over the last decade. The prevalence of co-resistance to more advanced cephalosporin (second, third and fourth generation), monobactams, piperacillin-tazobactam and aminoglycosides is increasing.[13] Hence regular monitoring of antibiotic resistance is crucial to prescribe appropriate therapy and also to prevent the spread of resistant strains in the hospital as well as in the community.[3, 15] The present study has been undertaken to evaluate the association of virulence factors with antibiotic susceptibility pattern of *E. coli* isolated from UTI in community and hospitalised patient population attending hospitals attached to Bangalore Medical College and Research Institute.

MATERIALS AND METHODS

This study was conducted from October 2010 to September 2012 in the Department of Microbiology attached to Victoria Hospital, Minto

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Institute of Ophthalmology and Vani Vilas Hospitals of Bangalore Medical College & Research Institute, Bangalore, India. Urine samples from suspected cases of UTI were processed by microscopy and culture. A total of 100 isolates of *E.coli* isolated from such cases were studied for the presence of virulence factors and antimicrobial susceptibility patterns.

Method of collection

The study population included both out patients and inpatients attending different clinical departments of Victoria and Vani Vilas hospital, BMC &RI with suspected UTI [i.e., with dysuria, frequency, urgency, fever] . The patients were asked to collect a midstream sample of urine in a sterile container. In case of catheterised patients the urine samples were collected after 30 minutes of clamping the catheter, through a syringe and needle inserted proximal to the site of clamping under all aseptic precautions. The specimens were immediately transported to the laboratory and processed.

Laboratory procedures

Semi quantitative culture for urine

A calibrated nichrome wire loop of 4mm diameter that delivers 0.01ml of urine was used to culture urine sample semi quantitatively. Urine sample was mixed thoroughly, the calibrated loop sterilized by red hot method was inserted vertically into the urine and a loopful of sample so removed was streaked on MacConkey's agar, Blood agar (BA) and CLED agar (Cysteine Lactose Electrolyte Deficient) agar.
The plated cultures were incubated at 37^0C overnight. The number of colonies grown on BA were counted and interpreted as CFU/ml next morning. The presences of 100 or more colonies were considered as Significant Bacteriuria. Lactose fermenting large, moist, smooth, colonies resembling *E coli* was identified by standard biochemical tests.

Identification[1, 2]

Lactose fermenting, motile, Gram negative bacilli were identified by the following biochemical tests as *E coli.*
Oxidase non producer, Catalase producer, Indole producer, Methyl Red + Voges Proskauer - , TSI agar showing A/A with gas and without H_2S
Urease not produced, Citrate not utilized. Glucose, Lactose, and Mannitol fermented with production of gas and Sucrose not fermented
Isolates were confirmed as *E coli* and antibiotic susceptibility testing was carried out.

Antibiotic susceptibility testing (AST)

AST was carried out by Kirby Bauer disc diffusion method on Mueller Hinton agar (Hi media). The media was prepared as per the instructions of the manufacturer by suspending 38g of dehydrated media in 1000ml of distilled water, autoclaved at 121^o C for 15 min and poured into petridishes to a depth of 4 mm. A broth culture of the isolate with turbidity adjusted to 0.5 McFarland turbidity standard was lawn cultured on the Mueller Hinton agar and allowed to dry. The antibiotic discs were taken out from the refrigerator brought to room temperature and were placed on the surface seeded with *E coli* and incubated at 37^oC overnight. The *E coli* isolates were tested for susceptibility to following antibiotics using commercial antibiotic disks (HiMedia).

Ampicillin (10 µg)
Cotrimoxazole (1.25 / 23.75 µg)
Amikacin (30µg)
Gentamicin (30µg)
Cefotaxime (30 µg)
Ofloxacin (5µg)
Norfloxacin (10µg)
Nitrofurantoin (300 µg)

The antibiotic susceptibility was interpreted as sensitive or resistant by comparing inhibitory zones produced by the test isolate with that of standard ATCC *E coli* 25922.

DETECTION OF VIRULENCE FACTORS

All fresh *E.coli* isolates from urine were tested for the following 3 virulence factors namely;

Hemolysin production
Cell surface hydrophobicity
Serum resistance

Hemolysin production[4,12]

The *E.coli* from urine were tested by plate hemolysis test. The bacteria were inoculated onto 5% sheep blood agar and incubated overnight at 35^0C.α Hemolysin production was indicated by the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.

Serum resistance[12,20]

Overnight cultures of *E coli* grown at 37°C on blood agar were harvested and cells suspended in Hanks Balanced Salt Solution (HBSS). Cell suspension (0.05ml) is mixed with fresh human serum (0.05ml) and 10µl of it was taken and plated on blood agar before incubation i.e., at 0 hour. The mixture was incubated for 3 hours and 10µl of it was plated out on blood agar. The plate was incubated for 18 -24 hours and number of colonies counted from them. Resistance of bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 3 hours of incubation with serum, in relation to count from 0 hour plate. Bacteria termed as sensitive if viable count dropped to 1% of initial value and resistant if ≥90 % of organisms survived after 3 hours.

Cell surface hydrophobicity[4,12,22]

The cell surface hydrophobicity was performed by salt aggregation test (SAT) using ammonium sulphate solution. Initially a 5M solution of ammonium sulphate was prepared from which different molar concentrations were prepared (i.e., 0.25, 0.5, 1.0, 1.25, 1.50, 2.0M). One loopful of different molar concentrations of ammonium sulphate was placed on VDRL slides. One loopful of bacterial suspension made in Phosphate buffer (pH 6.8) was added to the ammonium sulphate and mixed and rotated for one min. The highest dilution of ammonium sulphate solution that showed visible clumping was recorded as SAT value. The *E coli* that aggregated in phosphate buffer alone were considered as auto aggregative and not interpreted. *E coli* strains that showed SAT value of 1.4 and less were considered hydrophobic.

RESULTS

The present study was carried out on 100 *E coli* isolated from cases of UTI at the Department of Microbiology, BMC& RI from October 2010 to September 2012. All the *E.coli* from significant bacteruria were tested for three different virulence factors namely

1.Haemolysin
2.Cell surface hydrophobicity
3.Serum resistance

Antibiotic susceptibility testing was performed for all the isolates by Kirby – Bauer disc diffusion method.

The observations made from the study are shown in the following tables. A total of 100 patients with UTI were included in the study of which 45 were males and 55 were females. Maximum no of cases were seen between 1-9 years and 20-40yrs of age. Out of the 100 *E.coli* isolates tested for detection of Virulence factors 89% showed the presence of virulence factors while 11% tested negative for all 3 of them. Hence high incidence of virulence factors was noted among the *E.coli* isolates from UTI. Serum resistance was the most frequently observed virulence factor followed by Hemolysin production. Multiple virulence factors occurrence was more in UTI cases. No virulence factor was observed in 11% of cases. There was no significant difference in occurrence of virulence factors between males and females. Serum resistance was the most commonly observed virulence factor in children followed by Hemolysin production and presence of cell surface hydrophobicity. Maximum number of isolates showed sensitivity to Amikacin (87%) and Nitrofurantoin (81%) and resistance to Ampicillin (90%). Higher resistance to commonly used antibiotics like Ofloxacin (73%), Norfloxacin (67%) and cefotaxime (71%) was seen. It was observed that increased presence of virulence factors is associated with increase in antibiotic resistance.

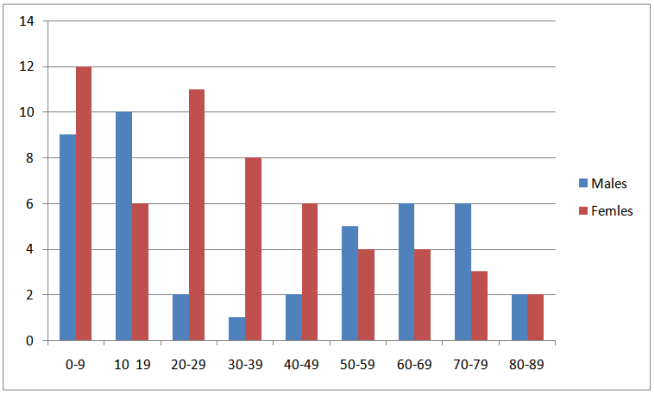

Figure 1: Age wise distribution of UTI cases

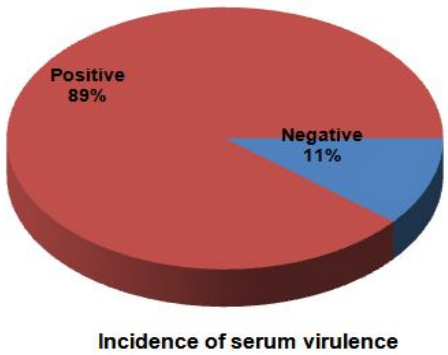

Figure 2: Incidence of serum virulence based on three test criteria

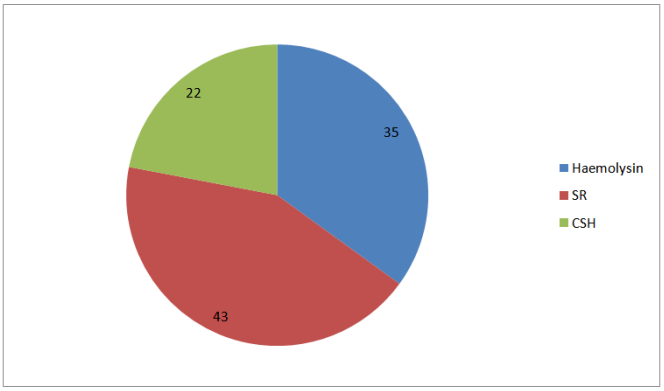

Figure 3: Virulence factor distribution

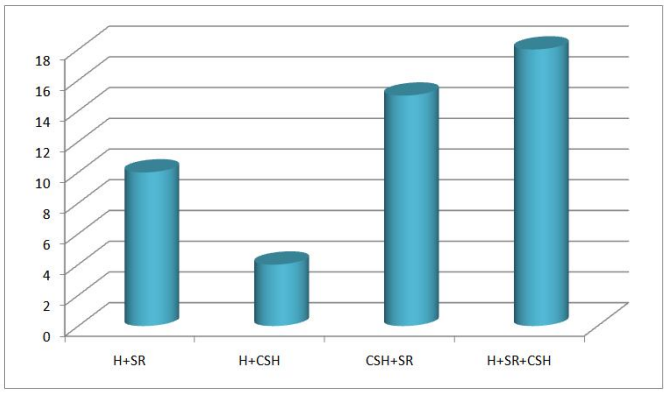

Figure 4: Occurrence virulence factors in combination

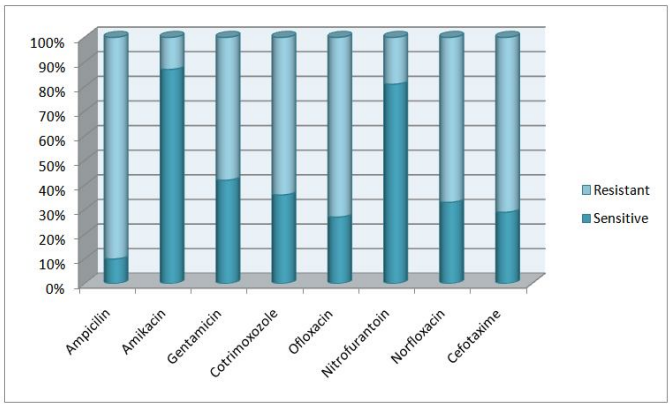

Figure 5: Antibiotic susceptibility pattern of *E.coli*

Table 1: Correlation of resistance and Sensitivity according to incidence of serum virulence

Antibiotics	Serum Virulence			
	Negative (n=11)		Positive (n=89)	
	Resistance	Sensitivity	Resistance	Sensitivity
1.Ampicillin	10(90.9%)	1(9.1%)	80(89.9%)	9(10.1%)
2.Co trimoxozole	7(63.6%)	4(36.4%)	57(64%)	32(36%)
3.Ofloxacin	7(63.6%)	4(36.4%)	66(74.2%)	23(25.8%)
4.Norfloxacin	9(81.8%)	2(18.2%)	58(65.2%)	31(34.8%)
5.Nitrofurantoin	3(27.3%)	8(72.7%)	16(18%)	73(82%)
6.Amikacin	3(27.3%)	8(72.7%)	10(11.2%)	79(88.8%)
7.Gentamycin	5(45.5%)	6(54.5%)	53(59.6%)	36(40.4%)
8.Cefotaxime	11(100%)	0(0%)	60(67.4%)	29(32.6%)

DISCUSSION

E. coli the most prevalent facultative Gram negative bacterium in the human fecal flora usually inhabits the colon as an innocuous commensal. It causes extra intestinal and intestinal infections.[10] UTI is the most common form of extra intestinal infection produced by *E.coli* and it is the most common cause of UTI. *E coli* is responsible for 50-90% of all uncomplicated UTIs.[17] These UTI causing *E coli* possess special properties called virulence factors to produce disease.[10] The treatment of *E coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by these organisms. From the study conducted it is revealed that large number of urinary isolates from UTI cases had more than one virulence factor. In the present study 89 out of 100 UTI isolates had one or more virulence factors, our results here correlate to Kausar et al who reported that 80% of the *E coli* from UTI had one or the other virulence factors. Combination of any two virulence factors was seen in 32.5% cases, three virulence factors was seen in 22% *E coli* from UTI. In the present study, *E coli* showed more resistance to commonly used antibiotics such as ofloxacin, norfloxacin, cephotaxime. There is also increasing resistance to Gentamicin. Ampicillin resistance was seen in 90% of cases. High resistance to

cephotaxime (71%) was seen. Amikacin resistance was seen in 13% of cases. In the present study the most sensitive drug is Amikacin with 87%.The least sensitive drug is Ampicillin with 10% . It was observed that virulence factors increased antibiotic resistance of resistant strains and increased the sensitivity of susceptible strains.
In view of the emerging drug resistance among *E.coli*, it is important to advocate therapy only after antibiotic sensitivity has been performed .By doing so we can not only prevent indiscriminate use of antibiotics but also the further development of antibiotic resistance.

Conclusion

UTI is the most common of bacterial infection both in community and hospitalized patients and *E.coli* is the most common bacterial agent causing it. In the present study 100 *E.coli* isolates from clinically suspected cases of UTI were studied along with the virulence factors like Serum resistance, Hemolysin production and Cell surface hydrophobicity. The methods of detection for the above discussed virulence factors are feasible. Serum resistance was the most frequently observed virulence factor followed by hemolysin and cell surface hydrophobicity. The majority of *E.coli* isolates were between the age group 0-9 yrs and 20- 40yrs.Among the 100 isolates 55% isolates were from female and 45 % from males. Amongst the 100 isolates 89% showed the presence of one or more virulence factors. Of the 89% *E.coli* 58% were serum resistant 46% haemolytic and 20% were positive for cell surface hydrophobicity. AST of the isolates showed maximum sensitivity to Amikacin followed by Nitrofurantoin, Gentamicin, Cotrimoxazole, Norfloxacin, Ofloxacin and least for Ampicillin. The methods of detection of the above mentioned virulence factors are easy for screening in routine Microbiology laboratory. Further studies on better understanding of interaction of different VF at molecular level are necessary as most urovirulent strains express multiple virulence factors simultaneously. In view of emerging drug resistance and multidrug resistance exhibited by *Escherichia coli*, periodic review and formulation of antibiotic policy are needed. Indiscriminate use of antibiotic must be discouraged and therapy should be advocated as for as possible after the culture and sensitivity reports are available.

Acknowledgements

The authors express gratitude to the faculty of the department of microbiology, Victoria Hospital, BMCRI, Bangalore for their valuable help in processing of clinical specimens for the present study.

BIBLIOGRAPHY

1. Chrichton PB. Enterobacteriaceae: *Escherichia, Klebsiella, Proteus* and other genera. In: Collee JG, Fraser AG, Marmion BP, Simmon A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th edition. Churchill Livingston: New York: 2006:361-84.
2. The *Enterobacteriaceae.* In: Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G editors. Koneman's Color atlas and textbook of diagnostic Microbiology. 6th edition. Philadelphia: Lippincott Williams & Wilkins: 212-302
3. Raksha R, Srinivasa H, Mecaden RS. Occurrence and characterization of Uropathogenic *E coli* in urinary tract infections. Indian J Med Microbiol. 2003; 21: 102-7.
4. Travis J Wiles, Richard R Kulesus, Mathew A Mulvey. Origin and Virulence Mechanism of UPEC. Experimental and Molecular Pathology; 2008; 85;11-19
5. Kausar Y, Chunchanur SK, Nadagir SD, Halesh LH, Chandrasekhar MR. Virulence factors, Serotypes and Antimicrobial susceptibility pattern of *Escherichia coli* in urinary tract infections. Al amen J Med Sciences.2009; 02:47-51.
6. J. Agarwal, S.Srivastava, M.Singh. Pathogenomics of Uropathogenic *Escherichia coli*. Indian J Med Microbiol 2012; 30 (2): 141-9.
7. Bhat GK, Sharma S, Shenoy S. Virulence factors and drug resistance in *Escherichia coli* isolated from extra intestinal infections. Indian J Med Microbiol. 2007; 25:369-73.
8. Johnson JR., Virulence factors in *Escherichia coli* urinary tract infections. Clin Microboli Rev 1991; 4:81-128.
9. Barclay L. Antibiotic resistance among Uropathogenic *E.coli* may be increasing. Annual conference on Antimicrobial resistance 2008; 23-25
10. Infections of the urinary tract. In: Forbes BA, Saham DF, Weissfeld AS editors. Bailey and Scott's diagnostic microbiology. 12th edition. Mosby Elsevier:2008;842-55
11. Steadman R, Topley N. The virulence of *Escherichia coli* in urinary tract. In: Brumfit W, Jeremy MT, Hamilton Miller editors. Urinary tract infections. 1st edition. Chapman and Hall: London: 1998: 37-41.
12. Siegfried L, Kmetova M, puzova H, Molokacova M, Filka J. Virulence associated factors in *E. coli* strains isolated from children with urinary tract infections. J Med Microbiol. 1994;41:127-32
13. Brauner A, Katouli M, Tullus K, Jacobson SH. Cell surface hydrophobicity, adherence to HeLa cell cultures and haemagglutination pattern of pyelonephritogenic *Esherichia coli* strains. Epidemiol Infect. 1990; 105:255-63.
14. Johnson JR., Virulence factors in *Escherichia coli* urinary tract infections. Clin Microboli Rev 1991; 4:81-128.
15. Vaisanen-Rhen, V, Elo J, Vaisanen E, Siitonen A, Orskov I, Orskov et al. P-fimbriated clones among uropathogenic Escherichia coli strains. Infect. Immun. 1984; 43:149-55.
16. Wang MC, Tseng CC, Chen CY et al. The role of bacterial virulence and host factors in patients with bacteremia who have acute cholangitis and upper urinary tract infection. Clin Infect Dis: 2002; 35:1161-66.
17. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, et al. Virulence Characteristics and Genetic Affinities of Multiple Drug Resistant Uropathogenic *Escherichia coli* from a Semi Urban Locality in India. PLoS ONE 2011; 6(3): e18063. doi:10.1371/journal.pone.0018063
18. Taylor PW. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol Rev 1983; 47:46-83.
19. Goldman RC ,Joiner K, Leive L. Serum-resistant mutants of *Escherichia coli* O111 contain increased lipopolysaccharide, lack an O antigen-containing capsule, and cover more of their lipid A core with O antigen. J Bacteriol 1984;159:877-882
20. Arisoy, Abbas Yusofi Rad, Ali Akin, Nejat Akar. Relationship between susceptibility to antimicrobials and virulence factors in paediatric *E coli* isolates. *Int J Antimicrobial Agents* 2008; 31 S4-8.
21. Naveen, Rebecca, Mathai, Elizabeth. Some virulence characteristics of Uropathogenic *E.coli* in different patient groups. Indian J of Medical Microbiol 2005 ;11-3
22. Kalpana Guptha, Delia Scholes, Walter E Stamm. Increasing prevalence of antimicrobial resistance among uropathogens causing cute uncomplicated cystitis in women. American Med Assoc 1999; 281(8): 736-38.
23. Chattarjee B, Kulathinal S, Bhargava A, Jain Y, Kataria R. antimicrobial resistance stratified by risk factor among *Escherichia coli* isolated from the urinary tract at a rural clinic in central India. Ind J Med Microbiol 2009; 27(4):329-34.

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