International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 11; November 2017; Page No. 7453-7456
DOI: http://dx.doi.org/10.24327/ijcar.2017.7456.1153

Research Article

PHENOTYPIC PROFILING OF VIRULENT ESCHERICHIA COLI ISOLATED FROM URINARY TRACT INFECTIONS FROM WESTERN DISTRICTS OF TAMIL NADU

Srinivasan Manivannan[1]* and Premkumar A[2]

[1]Research and Development Center, Bharathiar University, Coimbatore, India
[2]Pasteur Institute of India, Coonoor, India

A R T I C L E I N F O

Article History:
Received 18th August, 2017
Received in revised form 10th September, 2017
Accepted 06th October, 2017
Published online 28th November, 2017

Key words:
UTI, Antibiotic resistance, Phenotyping, *E.coli*.

A B S T R A C T

Objective: Urinary tract infection is a frequently encountered problem in developing and developed nations. There are various methods to identify UTI pathogens with different specificity and sensitivity. The study focuses on monitoring the prevalence of *E.coli* in patients with Urinary tract infections and UTI suspected patients.
Methods: The methods include preliminary processing of samples by culturing in BHI and screening of *E.coli* in EMB Agar. Further microbiological and biochemical characterization were carried out for identification.
Results: A predominant presence of pathogenic *E.coli* accounting for more than 50% of UTI was observed.
Conclusion: The results are indicative of an efficient screening and isolation method for *E.coli* in patients with UTI and UTI suspected patients. Moreover the antibiotic sensitivity screening patterns will aid in the treatment regime of the cases

INTRODUCTION

Escherichia coli is a common inhabitant of the gastrointestinal tract of humans and animals. Usually, *E. coli* forms a beneficial symbiotic relationship with its host and plays important roles in promoting the stability of the luminal microbial flora and in maintaining normal intestinal homeostasis (Yan and Polk, 2004). As a commensal, *E. coli* rather remains harmlessly confined to the intestinal lumen and rarely causes a disease. However, in the debilitated or immuno suppressed host, or when the gastrointestinal barriers are violated, even nonpathogenic-commensal strains of *E. coli* can cause infection (Kaper *et al*., 2004). Some strains of *E. coli* can diverge from their commensal cohorts, taking on a more pathogenic nature. These strains acquire specific virulence factors (via, DNA horizontal transfer of transposons, plasmids, bacteriophages, and pathogenicity islands) which confer an increased ability to adapt to new niches and allow the bacteria to increase the ability to cause a broad spectrum of diseases. Successful establishment of infection by bacterial pathogens requires adhesion to host cells, colonization of tissues, and, in certain cases, cellular invasion, followed by intracellular multiplication, dissemination to other tissues or persistence. Colonization of the urine in the absence of the clinical symptoms is called asymptomatic bacteriuria (ABU).

Corresponding author:* **Srinivasan Manivannan
Department of Biotechnology, Bharathiar University, Coimbatore, India

Most patients with ABU do not need treatment, and in many cases the colonizing by the ABU strains may help to prevent infection by other more virulent bacteria (Hull *et al*., 2000; Darouiche*et al*., 2001; Trautner *et al*., 2002). The primary causative agents responsible for more than 80% of all UTIs, including both ABU and symptomatic UTIs, are strains of uropathogenic *E. coli* (Hooton and Stamm, 1997; Svanborg and Godaly, 1997). Antibiotic treatment, typically with trimethoprim / sulfamethoxazole or ciprofloxacin, is generally effective for eradication of the infecting strain.

The current study is aimed at the incidence of UPEC despite previous studies since reports on UPEC in children are not available in this part of the state. UPEC strains encode a number of virulence factors, which enable the bacteria to colonize the urinary tract and persist in face of highly effective host defense. UPEC isolates exhibit a high degree of genetic diversity due to the possession of specialized virulence genes located on mobile genetic elements called pathogenicity islands (Oelschlaeger *et al*., 2002; Wiles *et al*., 2008). Attention has been given on the multiple antibiotic resistant and haemolysin positive strains of *UPEC* in clinical and diarrhoeal samples. In addition, the strains have been screened and characterized.

MATERIALS AND METHODS

Bacterial strain and isolates: A total of 137 urine samples (from patients having UTI and suspected UTI individuals) were collected from various hospital-attached laboratories and

private clinical laboratories located various places from the Districts of Coimbatore, Erode, Tiruppur, The Nilgiris and Salem. Collection of samples was done during the period from November 2014 to May, 2015. Throughout the study the standard strain of *E. coli* MTCC 326 was used as the positive control.

Growth and screening of UTI E. coli: All the urine samples were enriched in Brain heart infusion broth (BHIB) (HiMedia, India). 0.1 ml each samples were transferred to 99 ml of sterile BHI broth medium and incubated for 18 hrs at 37 ^{o}C. The resulting colonies were further streaked onto Eosine Methylene Blue (EMB) agar (HiMedia, India) and the samples showing green metallic sheen after 24 h of incubation were primarily identified as *E. coli*. Additionally, the isolates were analyzed for lactose fermentation by plating on MacConkey Agar (HiMedia, India).

Microbiological and Biochemical characterization: The green metallic colonies of *E. coli* were confirmed by Gram staining. Biochemical tests were performed according to Bergey's Manual of Determinative Bacteriology (Ref).

Antibiotic sensitivity test and MAR indexing: The antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusiontest as per Clinical and Laboratory Standard Institute (CLSI) guidelines. The isolates were tested forampicillin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), amoxicillin-clavulanic acid (10/20 µg), co-trimoxazole (1.25/23.75 µg), cefepime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg) and imipenem (10 µg) (Hi-media, Mumbai). An isolatewas considered as MDR if found resistant to three ormore antimicrobials belonging to different classes/groups of antimicrobials. Multiple antimicrobial resistance (MAR) profiles were also examined using β-lactam (17) and non β-lactam (42) antimicrobial agents. Bactericidal activity (serum susceptibility) of *E. coli* on normal human serum was also calculated.

RESULTS AND DISCUSSION

Bacterial strain and isolates

Table 1 Samples, sampling area and sample data

Month	Sampling area	No of urine samples		Total	Total
		UTI samples	Non-UTI samples		
	Coimbatore	6	1	7	
	Erode	2	0	2	
	Tirupur	0	1	1	
Nov-14	Kangeyam	1	0	1	22
	Annur	3	2	5	
	Salem	2	2	4	
	Ooty/Coonoor	1	1	2	
	Coimbatore	2	1	3	
	Erode	3	1	4	
	Tirupur	1	0	1	
Dec-14	Kangeyam	-	-	0	20
	Annur	3	1	4	
	Salem	4	2	6	
	Ooty/Coonoor	2	0	2	
	Coimbatore	2	0	2	
Jan-14	Erode	3	1	4	19
	Tirupur	2	1	3	
	Kangeyam	--	--	--	
	Annur	2	3	5	
	Salem	3	0	3	
	Ooty/Coonoor	2	0	2	
	Coimbatore	15	-	15	
	Erode	2	1	3	
	Tirupur	6	0	6	
Feb-14	Kangeyam	3	0	1	27
	Annur	1	1	2	
	Salem	--	--	--	
	Ooty/Coonoor	--	--	--	
	Coimbatore	4	1	5	
	Erode	1	2	3	
	Tirupur	3	0	3	
Mar-14	Kangeyam	4	1	5	24
	Annur	-	1	1	
	Salem	2	2	4	
	Ooty/Coonoor	3	0	3	
	Coimbatore	1	-	1	
	Erode	0	0	0	
	Tirupur	4	0	4	
Apr-14	Kangeyam	2	1	3	12
	Annur	1	1	2	
	Salem	0	1	1	
	Ooty/Coonoor	1	0	1	
	Coimbatore	2	1	3	
	Erode	3	1	4	
	Tirupur	1	2	3	
May-14	Kangeyam	3	0	3	13
	Annur	0	0	0	
	Salem	0	0	0	
	Ooty/Coonoor	0	0	0	
	Total	106	33		137

All the specimens were labeled as UTE 01 to UTE 106; NUT 01 to 33

Growth and screening of UTI E. coli

Total No. of samples	Positive Green metallic sheen colony			
	UTI samples (n=106)	% of incidence	Non UTI samples (n=33)	% of incidence
137	57	53.77	8	24.3

Percentage incidence of LF and NLF bacterial growth on MacConkey agar

No. of Samples Collected	UTI samples		Non UTI Samples	
	LFB*+ve	%	LFB*+ve	%
137	59	55.66	16	48.48

LFB* - Lactose fermenting bacteria; **NLFB**** - Non Lactose fermenting bacteria

CONCLUSION

The result of the present study shows a prevalence and distribution of *E.coli* in patients with UTI and suspected UTI. Further studies on the virulence and pathogenicity of the strains are proposed to be carried out to better understand the role of pathogenic *E.coli* in the disease pathogenesis of UTI, since pathogens related to these infections are gaining importance due to sudden outbreaks.

Microbiological and Biochemical characterization

Biochemical Tests	*E. coli*	UTE																			
	MTCC 326	*1*	*2*	*3*	*4*	*6*	*7*	*8*	*10*	*11*	*13*	*14*	*16*	*18*	*19*	*20*	*24*	*25*	*26*	*29*	*30*
Colour (on EMB)	Metallic sheen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram-stain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cytochrome oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ONPG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate-Nitrite	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid (-A) and Gas (-G) from:																					
Arabinose	A	A	A	A	A	AG	A	A	A	A	A	AG	A	A	A	AG	A	A	A	A	A
Cellobiose	AG	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Fructose	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Galactose	A --	A --	A --	AG	A --	A --	A --	A G	A --	A --	A --	A --	A --	A --	A --	A --	A --	A --	A --	A --	A -
Glucose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Maltose	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mannitol	--	--	--	--	--	--	--	A	--	--	--	G	--	--	--	--	--	--	--	G	--
Mannose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	
Sorbitol	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	

Antibiotic sensitivity test and MAR indexing: The antibiotic sensitivity testing revealed normal sensitivity and some resistant patterns. The results were arrived with the comparison of MTCC 326 as a standard.

References

1. Darouiche, R. O., W. H. Donovan, M. Del Terzo, J. I. Thornby, D. C. Rudy, and R. A. Hull, "Pilot trial of bacterial interference for preventing urinary tract infection," *Urology*, vol. 58, no. 3, pp. 339-344, 2001.
2. Emödy, L., M. Kerényi, and G. Nagy, "Virulence factors of uropathogenic *Escherichia coli*," *International Journal of Antimicrobial Agents*, vol. 22, no. 2, pp. S29-S33, 2003.
3. Foxman, B., "Epidemiology of urinary tract infections: incidence, morbidity, and economic costs," Disease-a-Month, vol. 49, no. 2, pp. 53-70, 2003.
4. Hooton T. M. and W. E. Stamm, "Diagnosis and treatment of uncomplicated urinary tract infection," *Infectious Disease Clinics of North America*, vol. 11, no. 3, pp. 551-581, 1997.
5. Hull, R., D. Rudy, W. Donovan et al., "Urinary tract infection prophylaxis using *Escherichia coli* 83972 in spinal cord injured patients," *Journal of Urology,* vol. 163, no. 3, pp. 872-877, 2000.
6. Kaper, J. B., J. P. Nataro, and H. L. T. Mobley, "Pathogenic *Escherichia coli*," Nature Reviews Microbiology, vol. 2, no. 2, pp. 123-140, 2004.
7. Manges, A. R., J. R. Johnson, B. Foxman, T. T. O'Bryan, K. E. Fullerton, and L. W. Riley, "Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group," *New England Journal of Medicine*, vol. 345, no. 14, pp. 1007-1013, 2001.
8. Martinez, J. J., M. A. Mulvey, J. D. Schilling, J. S. Pinkner, and S. J. Hultgren, "Type 1 pilus-mediated bacterial invasion of bladder epithelial cells," *EMBO Journal*, vol. 19, no. 12, pp. 2803-2812, 2000.
9. Mobley H. L. T., and J. W. Warren, Urinary Tract Infections: Molecular Pathogenesis and Clinical Management, ASM Press, Washington, DC, USA, 1996.
10. Mulvey, M. A., Y. S. Lopez-Boado, C. L. Wilson et al., "Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*," *Science*, vol. 282, no. 5393, pp. 1494-1497, 1998.
11. Nicolle, L. E. "Urinary tract infection in geriatric and institutionalized patients," Current Opinion in Urology, vol. 12, no. 1, pp. 51-55, 2002.
12. Oelschlaeger, T. A., U. Dobrindt, and J. Hacker, "Pathogenicity islands of uropathogenic E. coli and the evolution of virulence," *International Journal of Antimicrobial Agents*, vol. 19, no. 6, pp. 517-521, 2002.
13. Russo, T. A. and J. R. Johnson, "Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC," *Journal of Infectious Diseases,* vol. 181, no. 5, pp. 1753-1754, 2000.
14. Russo, T. A., A. Stapleton, S. Wenderoth, T. M. Hooton, and W. E. Stamm, "Chromosomal restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women," *Journal of Infectious Diseases*, vol. 172, no. 2, pp. 440-445, 1995.
15. Svanborg C. and G. Godaly, "Bacterial virulence in urinary tract infection," Infectious Disease Clinics of North America, vol. 11, no. 3, pp. 513-529, 1997.
16. Trautner, B. W., R. A. Hull, and R. O. Darouiche, "*Escherichia coli* 83972 inhibits catheter adherence by a broad spectrum of uropathogens," *Urology*, vol. 61, no. 5, pp. 1059-1062, 2003.
17. Wiles, T. J., R. R. Kulesus, and M. A. Mulvey, "Origins and virulence mechanisms of uropathogenic

Escherichia coli," *Experimental and Molecular Pathology*, vol. 85, no. 1, pp. 11-19, 2008.

18. Yan, F. and D. B. Polk, "Commensal bacteria in the gut: learning who our friends are," Current Opinion in Gastroenterology, vol. 20, no. 6, pp. 565-571, 2004.

How to cite this article:

Srinivasan Manivannan and Premkumar A(2017) 'Phenotypic Profiling of Virulent Escherichia Coli Isolated From Urinary Tract Infections From Western Districts of Tamil Nadu', *International Journal of Current Advanced Research,* 06(11), pp. 7453-7456. DOI: http://dx.doi.org/10.24327/ijcar.2017.7456.1153

****