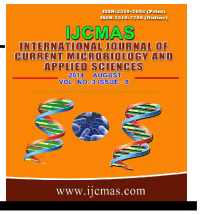
Int.J.Curr.Microbiol.App.Sci (2014) 3(8) 823-828

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 823-828**
http://www.ijcmas.com



Original Research Article

Serum Bactericidal Resistance in Uropathogenic *E.coli*

Sabitha Baby[1], Vimal Kumar Karnaker[2*] and R.K.Geetha[1]

[1]Department of Microbiology, Karuna Medical College, Chittur, Kerala, India
[2]Department of Microbiology, KS HegdeMedical Academy, Nitte, Mangalore, India
**Corresponding author*

Keywords

E.coli, serum resistance, UPEC

A B S T R A C T

Urinary tract infections (UTIs) are caused by *E.coli* and account for about 80% of community accquried UTI. In this study the bactericidal resistance of serum by Uropathogenic *E.coli* (UPEC) was compared with the resistance shown by intestinal isolates (control group) obtained from fecal specimen. A total of 300 UPEC and 30 intestinal *E.coli* were tested.

Introduction

Urinary tract infections(UTIs) are among the most common bacterial infections encountered in clinical practice (Ejrnaes, 2011)) *E.coli* causes about 80%of acute UTIs(Mitsumori,1998).*E.coli* is the most prevalent facultative gram negative bacilli in the human fecal flora, usually inhabits the colon as an innocuous commensal (Johnson, 1991). *E.coli* strains causing disease outside the gastrointestinal tract have been named extra intestinal pathogenic *E.coli* (ExPEC). Among ExPEC, UPEC is the most common pathogen in humans (Giray *et* al,2012).

Urinary infections most commonly occur in patients with anatomically and functionally normal urinary tract and involves spontaneous ascent of bacteria from the urethra to the bladder and to the kidneys and blood stream (Fathollahi *et al*,2009).

The UTIs comprise of a range of disorders, including cystitis (infections of the bladder) and pyelonephritis (infection of the kidney)with the possibility of causing irreversible kidney damage (Kulkarni *et* al, 2009).

Virulence refers to the degree of pathogenicity of an organism or in other words the relative ability of a pathogen to cause disease. Virulence factors(VFs)are specific properties that enable organisms to overcome host defenses and cause diseases.(Johnson *et al*, 1991).UPEC have special subset of virulence factors that enable them to colonise periurethral area and enter urinary tract. UPEC have the ability to adhere to uroepithelial cells. They also have O and K antigens which make them resistant to phagocytosis and to the bactericidal

action of normal serum. Other factors known to contribute the virulence are the production of alpha hemolysins, colicins,aerobacter, cytotoxic necrotising factor and cell surface hydrophobicity(Raksha *et al*,2003)

The virulence of UPEC is multifactorial.The pathogenic processes that operate in a given infection involve more than one virulence factor. The factors usually interact into a complicated manner that the precise mechanism still remains to be defined.(Bhatt *et al* ,2006).Besides bacterial adhesins other important mechanism include the host defense avoidance mechanisms(Tiba *et* al,2008).Serum resistance is an important virulence marker in UPEC But the prevalence with respect to commensal gastrointestinal strains is less studied.

The aim of this study was to compare the prevalence of serum resistance of UPEC with intestinal strains (control group) from stool samples of healthy individuals. The UPEC were from patients with UTI, from a tertiary hospital in Kerala. The lethal effect of human serum for Gram negative bacteria is well recognised (Dickson *et al*1980).The bactericidal activity is mediated by activation of complement system, which activity is lost when complement is inactivated or scanty.The ability to overcome the bactericidal action of serum will confer survival advantage to UPEC in the bladder

Chi-square test was carried out to determine the statistical significance, while comparing the serum resistance of UPEC and control strains

Materials and Methods

This study was conducted in the Karuna Institute of Medical Sciences, Chittur, A total number of 300 *E.coli* isolates were obtained from 300 urine samples (cases) and 30 stool samples (controls) over a period of two years (August 2011-July 2013) from various department in our hospital. Ethical clearance has been obtained from the institution.

The inclusion criteria were *E.coli* which was isolated from the urine that had significant bacteriuria and the stool samples were from healthy individuals.The patients who were on a current antibiotic therapy were excluded from study.

Sample collection

Mid-stream clean catch urine, catheterized urine and stool samples were collected in sterile containers which were labelled with the patients' details. The specimens were transported to the laboratory in leak proof boxes and they were processed as soon as possible. When the processing was delayed, they were stored at 4°C.

Specimen processing

The urine samples were observed macroscopically for their colour and turbidity. Wet mounts of the samples were prepared and examined for the presence of pus cells and organisms.

Standard techniques were used to identify the *E.coli* isolates.Semi quantitative cultures were done by inoculating thoroughly the mixed urine onto a 5% sheep blood agar plate and on a Mac Conkey's agar plate with a calibrated loop. The inoculated plates were incubated at 37 °C overnight.The UPEC isolates were stored in LB broth ,until analysis.

Serum obtained from healthy humans, who were not on antibiotics.The serum was

pooled and stored in aliquots under refrigeration, until required.Control serwas prepared by heating the serum at 56^0C for 30 minutes and inactivating the complement.

Serum Bactericidal Assay

This assay done according to Siegfried *et al.*Overnight cultures of *E. coli,* grown at 37°C on Muller Hinton Agar (MHA), is harvested and the cells were suspended in Hanks's Balanced Salts Solution (HBSS) . Test tubes were used for incubation of bacterial suspensions (0.05 ml) with serum (0.05 ml). Control wells contained 0.05 ml of HBSS instead of serum. The tubes were mixed by shaking for a minute and incubated at 37^0C in an incubator. Samples (10 µl) were taken at 0 minute and after incubation for180 min at 37°C and spread on MHA. The plates were further incubated for 18 h at 37°C. Susceptibility of bacteria to serum bactericidal activity expressed as the percentage of bacteria surviving after 180 min and overnight incubation in relation to the original count of bacteria determined at 0 min in the controls. According to Benge *et al* Strains termed serum sensitive if the viable count dropped to 1% of the initial value and resistant if> 90% of organisms survived after 180 min and overnight incubation. Strains that gave results between these values were considered to show intermediate sensitivity.An isolate that showed resiatnce to serum repeatedly was used as positive control

The results were analyzed by *Chi* square test for comparing the serum resistance pattern between test and control strains. A *p* value of ≤0.05 was taken as significant.

Results and Discussion

Among the 300 UPEC isolates 63.5 % showed resistance to the bactericidal action of the serum.While in the control group 20% showed serum resistance. There was no notable difference in the growth pattern of the test and control strains during zero minute of incubation. It was 100% growth. After three hours (180 minutes) the growth rate dropped to 35.5% in test strains and 80% in control group. No growth was noticed among the 63.5% isolates in UPEC as their viable count dropped after 180minutes . In control strains about 20%were showing resistance to bactericidal effect of serumThe growth rate in UPEC and control strains are shown in *Table 1*.The difference in serum resistance between UPEC and control strains were statistically significant as *p* value was 0.001.

In this study of the 300 UPEC strains 63.5%showed serum bactericidal effect. Among the control group of 30 fecal isolates 20%showed serum resistance. In these isolates there was no decrease in the viable count after incubating with serum .More *E.coli* isolates from UPEC were resistant to serum killing as compared to the control group. And this difference was statistically significant. Bacteria as earlier mentioned, are killed by normal serum through.lytic activity of the alternative complement pathway (Jadhav *et al*, 2011). About 16%showed intermediate resistance while20.5% were sensitive to serum and showed no growth among the UPEC cases.The intestinal isolates which formed the control showed serum sensitivity for about 70%and in this group 10% showed intermediate resistance.

The alternate complement pathway activation is the significant step-in serum resistance. In serum resistance pathogen gains the ability to resist the bactericidal activity of serum. This characteristic in pathogenesis enable bacteria to persist in body fluids and internal organs. Bacterial

resistance to killing by serum results due to capsular polysaccharides , surface proteins and lipopolysaccharides (Lefler *et al*, 1981).This study results were similar to Seigfred *et* al (1992) were 68% UPEC showed resistance. In the study of Sharma *et* al (2007), 86% of UPEC had shown resistance to serum. While Prachi *et* al (2012) reported 60% resistance among *E.coli.* This is in contrast to Raksha *et* al(2003) were UPEC showed only 32.7% resistance. While in studies of Kauser *et* al exhibited 32% resistance.

Group two and three capsular polysaccharides have been associated with UPEC and have been speculated to be important in UTI pathogenesis (Johnson et al , 2005).On the basis of te investigations performed we can assume that among the E.coli causing UTI,serum resistance is a critical virulence marker.The capsular polysaccharide helps in overcoming the complement cascade of the serum which will help the UPEC to invade the bladder and cause UTI.

The draw back of our study was that only single method using culture was adapted.Further genotypic and mutant studies shall help to determine the exact role of serum resistance in the pathogenesis of UTI.

In *E.coli* causing urinary tract infections, serum resistance is a significant virulence matter.Screening for serum resistance is relatively easy and can be carried out. The data generated shall help, for better understanding of the mechanism of serum resistance .Further genotyping and *in vivo* studies are necessary in this regard. This will on a wider scale help in acquiring better knowledge in the steps of disease pathogenesis and to find suitable vaccine candidates.

Table.1 Serum Bactericidal Resistance in intestinal and Uropathogenic *E.coli*

Viable count	%(percentage)					
	Growth at 0 minute		growth at 180 minutes		overnight incubation	
	control	UPEC	control	UPEC	control	UPEC
100%	100	100	20	63.5	20	63.5
50%	0	0	10	16	10	16
No growth	0	0	70	20.5	70	20.5
						P=≤0.001

References

Barron EJ, Fingold MS Baily, Scott, editors. 1990. Diagnostic Microbiology. 8th ed. Mosby Public Philadelphia Toronto, Baltimore 259–260

Benge GR.1988.Bactericidal activity of human serum against strains of Klebsiella from different sources. J Med Microbiol;27.1.:11–15

Bhat G K, Mallya S, Hegdae A .2008. Effect of exposure to hydrogen peroxide on the virulence of *Escherichia coli* IJMM: 26 : 1 : 25-28

Binns MM, Mayden J and Levine RP .1982.. Further characterization of complement resistance conferred on

Escherichia coli by the plasmid genes traT of R100 and is of ColV,I-K94. Infect. Immun.35: 654-659.

Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A Collee JG, Fraser AG, Marmion BP, Simmons A, editors. .1996. Laboratory strategy in the diagnosis of infective syndromes. Mackie and McCartney practical medical microbiology. 14th ed 53–94. Churchill Livingstone Inc: London.

Ejrnæs K.,2011 .Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*,Danish medical bulletin 58:4 ;4187

Fathollahi1 S, Yousefi-M R, Goodazi1T M ,Hajilooei1M, Hemati M,Mostafaei A, Sadeghian1 S ,.2009. Typing of the uropathogenic E.coli strains usingO-serotyping and detection of pap adhesion-encodingoperon by polymerase chain reaction.Iranian Journal of Clinical Infectious Diseases*;4.2.:77-81*

Giray B Füsun B U Ayedemir SS , 2012 Characterization of uropathogenic Escherichia coli strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir Turk J Med Sci; 42.Sup.1.: 1328-1337

Hughes C, Phillips R, Roberts AP.1982. Serum resistance among Escherichia coli strains causing urinary tract infection in relation to O type and the carriage of hemolysin, colicin, and antibiotic resistance determinants. Infect Immun.;35.1.:270–275

Jadhav S, Hussain A Devi S ,Kumar A, ParveenS GandhamN , Wieler L, EwersC, Ahmed N .2011. Virulence Characteristics and Genetic Affinities of Multiple Drug Resistant Uropathogenic *Escherichia coli* from a Semi Urban Locality in India DOI: 10.1371/journal.pone.0018063

Johnson JR,.1991. Virulence factors in Escherichia coli urinary tract infection.Clinical Micro review Jan;4.1.:80-128

Johnson Jr, Russo TA ,.2005. Molecular epidemiology of extraintestinal pathogenic .uropathogenic. Escherichia coli Int J Med Microbiol. Oct;295.6-7.:383-404.

Johnson JR .2003. Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect Dis clin North Am 17.2.: 261–71.

KausarY,Chunchanur S, Nadagir S,Halesh LH, Chandrasekhar M R.2009.. The virulence factors, serotypes and the antimicrobial susceptibility pattern of Escherichia coli in urinary tract infections. Al Ameen J Med Sci. 2.1.:47-51.

Kulkarni R,Dhakal BK ,Slechta,SA, Kurtz Z, MulveyMA , Thanassi D,.2009.Roles of Putative Type II Secretion and Type IV Pilus Systems in the Virulence of Uropathogenic *Escherichia coli PLoS one* March 09, DOI: 10.1371/journal.pone.0004752

Kubens BS, Wettstein M, Opferkuch W.1988. Two different mechanisms of serum resistance in Escherichia coli. Microb Pathog. Nov;5.5.:371–379.

Leffler H, Svanborg-Eden C .1981. Glycolipid receptors for uropathogenic Escherichia coli on human erythrocytes and uroepithelial cells. Infect Immun 34: 920–929

Mitsumori K[1], Terai A, Yamamoto S, .1998. Identification of S, F1C and three PapG fimbrial adhesins in uropathogenic Escherichia coli by polymerase chain reaction.FEMS ; Aug;21.4.:261-8

Mellata M, Dho-Moulin M, Dozois CM, Curtiss R, III, et al. .2003.. Role of virulence factors in resistance of avian

pathogenic Escherichia coli to serum and in pathogenicity. Infect. Immun.71: 536-540.

Najar GA, Nejad MM, Mansouri S .2007. The comparison between virulence factors of *Escherichia coli* isolated from urinary tract infections and fecal flora. Res Pharm Sci 2: 99–103.

Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, Surek M, Picheth G, Fadel-Picheth CM..2011. Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains.Gen Mol Res.Oct 31;10.4.:4114-25. doi: 10.4238/ 2011.October.31.5

Raksha, R ,SrinivasaH, Macaden R S.2003. Occurrence and characterisation of uropathogenic escherichia coli in urinary tract infections, IJMM21 : 2: 102-107

Prachi S, Chandan S, Ssaileela K.2012. Pattern Of Serum Resistance In Uropathogenic Escherechia Coli Intl Jrnl Pharm Bio Scie 3:April-June

Pezzlo M, York MK .2004. Urine Cultures. In: Isenberg HD, editor. Clinical Microbiology Procedures Handbook 2nd ed. Washington D. C.: American Society of Microbiology. pp. 31211–31231.

Puzova H, Siegfried L, Kmetova M, Filka J, Takacova V, et al. .1994. Fimbriation, surface hydrophobicity and serum resistance in uropathogenic strains of Escherichia coli. FEMS Immunol Med Microbiol 9: 223–229.

RakshaR , Srinivasa H ,MacadenR S.2003. Occurrence and characterisation of uropathogenic escherichia coli in urinary tract infections Ind Jrnl Med Micro21 :2: 102-107

Rebecca N, Elizabeth M, Mathai N.2005. Some virulence characteristics uropathogenic Escherichia coli In different patient groups Ind JMed Res 122,Aug:143-147

Sharma, S., *et al.* 2007.Expression of Two Recombinant Chloramphenicol Acetyltransferase Variants in Highly Reduced Genome Escherichia coli Strains. Biotechnology and Bioengineering, Vol. 98, No. 5

Siegfried L, Kmetova M, Puzova H, Molokacova M, Filka J .1994. Virulence-associated factors in Escherichia coli strains isolated from children with urinary tract infections. J Med Microbiol 41: 127–132

Tiba MS ,Yano T LReite D S.2008. ,Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. ev. Inst. Med. trop. S. Paulo vol.50 .5.