academicJournals

Vol. 12(9), pp. 230-236, 7 March, 2018
DOI: 10.5897/AJMR2017. 8771
Article Number: 2B8062956221
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Genotypic detection of the virulence factors of uropathogenic *Escherichia coli* isolated from diarrheic and urinary tract infected patients in Khartoum State, Sudan

Husam Eldin M. Hassan[1], Hisham N. Altayb[1], Mogahid M. El Hassan[2] and Miskelyemen A. Elmekki[2*]

[1]College of Medical Laboratory Science, Sudan University of Science and Technology, Sudan.
[2]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taibah University, KSA.

Received 24 November, 2017; Accepted 15 January, 2018

This study aimed to identify some important virulence factors, including *pap, fim, sfa, aer* and *hly* genes, typical of uropathogenic *Escherichia coli* (UPEC) in isolates collected from diarrheic and urinary tract infected patients in Khartoum State by multiplex polymerase chain reaction (PCR) assay. A total of 100 clinical specimens (50 urine and 50 diarrhea) were collected. Samples were cultured and identified by conventional method. Most study population were females 57/100 (57%); 42 suffering from urinary tract infections (UTIs) and 15 from diarrhea, while males were 43/100 (43%); 8 suffering from UTIs and 35 from diarrhea. Among enrolled subjects, 83 were positive for one or more uropathogenic *E. coli* virulent genes, while 17 isolates were negative for all genes. The results of multiplex PCR revealed that thirty two (n=32) diarrheal samples and fourteen (n=14) urine samples were *aer* positive. Thirty three (n=33) urine samples and eight (n=8) diarrheal samples appeared as *fim* positive. The genes *pap* and *hly* were found in 24 and 14 urine samples, respectively and in 9 and 3 diarrheal samples, respectively, while *sfa* gene was detected only in 15 urine specimens. The study concluded that *fim* gene was highly prevalent among UTI patients while *aer* gene was highly prevalent among diarrhea patients.

Key words: Uropathogenic *Escherichia coli*, *fimH*, aer, pap, sfa, hly, Sudan.

INTRODUCTION

Escherichia coli are a genetically diverse species that includes many pathotypes, both intestinal and extra-intestinal, most of which own specialized mechanisms characterized by their high efficiency in both colonization and pathogenicity. The appearance of different bacterial pathotypes is mainly due to horizontal transfer and exchange of genes responsible for virulence (Johnson, 2002).

E. coli possess genes encoding many pathogenicity associated factors including adhesions, siderophores

*Corresponding author. E-mail: mmekki@taibahu.edu.sa, miskatti@yahoo.com.

(that is, aerobactin), capsule and toxins implicated in urinary tract infection (UTI) pathogenesis. Several pathogenicity-associated genes were identified after the publication of the sequence of the whole genome of strain CFT073, a uropathogenic *E. coli* (UPEC). This strain was identified as the most cytotoxic that cause acute pyelonephritis as it was found to cause cytotoxicity in tissue culture cells in less than 18 h. Another strain that has been identified as invasive, the uropathogenic *E. coli* strain 536 (O6:K15:H31), which was isolated from acute pyelonephritis patient, this strain possess certain virulence factors; hemolysin (hly), P-related and S fimbriae (fim, prf, sfa), fimbrial adhesins type 1, the siderophores enterobactin (ent) and yersiniabactin (ybt) (Mobley et al., 1990). Moreover, acquisition of PIAs through horizontal gene transfer was also identified (Welch et al., 2002). Regardless of the common presence of type 1 fimbriae among Enterobacteriaceae, type 1 fimbriae may increase the virulence of UPEC for the urinary tract infection through several mechanisms including the promotion of bacterial persistence as well as enhancing the inflammation (Wullt, 2003).

Toxins produced by UPEC include hemolysin, cytotoxic necrotizing factor 1 (CNF1) and secreted auto transporter toxin (Sat) which has been shown to have a cytopathic effect on various bladder and kidney cell lines (Bahrani-Mougeot et al., 2002). In strain 536, four PAIs have been characterized which carry many pathogenicity associated genes (Dobrindt et al., 2002). The K15 capsule determinant of UPEC strain 536 is also found on a PAI (PAI V536) (Schneider et al., 2004). Some PAIs show genetic instability, whereas others appear to be relatively stable (Middendorf et al., 2004).

Some strains of *E. coli* considered as a common source of UTI and are able to colonise the vagina and found to originate from the lower GI tract (Czaja et al., 2009; Obata-Yasuoka et al., 2002). These strains usually originate from the stool, colonise the vagina and the periuretheral area through which they enter the UT (Salyers and Whitt, 2002). The presence of these bacteria in the UT without clinical symptoms is known as bacteriuria (Johnson, 1991; Mabbett, 2009). *E. coli* strains that colonise the UT may ascend towards bladder to cause cystitis and most probably, pyelonephritis, that may lead to kidney failure and death (Scholes et al., 2005).

The most accepted theory today is that UPEC germinated from non-pathogenic strains by gaining new virulence factors via accessory DNA horizontal transfer often organized into clusters (pathogenicity islands) located at chromosomal locus (Bahalo et al., 2013). The most common operons encoding P, S fimbriae are pyelonephritis associated pili (*Pap*) and S fimbrial adhesion (*sfa*) (Ribeiro et al., 2008). The pathogenicity of UPEC can be mediated by several virulence factors including bacterial adherence, in addition to the production of hemolysin and aerobactin (Arisoy et al., 2005). This study was performed to determine the virulence factors of UPEC in Khartoum.

MATERIALS AND METHODS

One hundred *E. coli* isolates were obtained from 100 clinical samples (50 urine, 50 diarrhea) collected from patients attending different hospitals in Khartoum state complaining from urinary tract infection and diarrhea, in the period from March to May, 2017. Urine samples were cultured on CLED agar, while diarrhea samples were cultured on MacConkey agar. Biochemical tests including indole as a key test, then urease, citrate and Kliger Iron Agar (KIA) test (according to CLSI guidelines) were used for identification of bacteria, in addition to the lactose fermentation on the MacConkey agar (Bahalo et al., 2013).

Bacterial isolates were grown on nutrient agar for an overnight then used for DNA extraction by boiling method (Yamamoto et al., 1995).

Polymerase chain reaction

Specific primers from Macrogen (Korea) were used to amplify the *fimH, pap, sfa, hly* and *aer* genes as shown in Table 1. The multiplex PCR assay was carried out in a total volume of 25 µL of mixture containing 2 µL Maxime PCR Premix (iNtRON, Korea) containing 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, and 1 U Taq DNA polymerase, 0.5 µL of each of the virulence gene-specific primers, forward and reverse primers for each gene (a total of 5 µL for the 5 target genes), 2 µL of template DNA and 16 µL of deionized water. The amplification conditions included three steps: heating at 94°C for 3 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and the final extension at 72°C for 7 min (Jalali et al., 2015). Amplification was done using TECHNE® Ltd. peltier thermal cycler (Germany).

Visualization of the PCR products

The PCR product was visualized on 1.5% agarose gel in TBE buffer, 100-bp DNA ladder (iNtRON, Korea) was used to determine product size.

Data analysis

Statistical Package for Social Science Program (SPSS) version (11.5) was used for data analysis, *p*-value of less than 0.05 was considered significant (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp, Released, 2012).

RESULTS

A total of 100 patients (50 patients suffering from UTIs and 50 patients suffering from diarrhea) attending Khartoum hospitals during March to May, 2017, were enrolled in this study. Table 2 shows the association between UPEC virulence genes and gender while the association between UPEC virulence genes and the type of clinical specimen is shown in Table 3. The results of agarose gel electrophoresis for multiplex PCR are as shown in Figure 1A and B.

Table 1. Primers used for detection of virulence genes of UPEC strains.

Identified gene	Primer	Primers sequence (5'-3')	Product size (bp)
papE/F	*pap3* *pap4*	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	336
fimH	*fim1* *fim2*	GAGAAGAGGTTTGATTTAACTTATTG AGAGCCGCTGTAGAACTGAGG	508
sfaD/E	*sfa1* *sfa2*	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410
Aer	*aer1* *aer2*	TACCGGATTGTCATATGCAGACCGT AATATCTTCCTCCAGTCCGGAGAAG	602
hlyA	*hly1* *hly2*	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177

Jalali et al., 2015.

Table 2. Association between the presence of UPEC virulence genes and gender.

Gene	*pap*		*fimH*		*sfa*		*aer*		*hly*		Total
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
Male	11	32	11	32	3	40	21	22	6	37	43
Female	22	35	30	27	12	45	25	32	11	46	57
Total	33	67	41	59	15	85	46	54	17	83	100
P-value	0.171		0.006		0.051		0.621		0.481		-

Table 3. Frequency of UPEC virulence genes in urine and diarrhea samples.

Gene	*pap*		*fimH*		*sfa*		*aer*		*hly*		Total
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
Urine	24	26	33	17	15	35	14	36	14	36	50
Diarrhoea	9	41	8	42	0	50	32	18	3	47	50
Total	33	67	41	59	15	85	46	54	17	83	100
P-value	0.001		0.000		0.000		0.000		0.003		-

There was significant association between the presences of *pap* gene and gentamicin, ciprofloxacin and co-trimoxazole susceptibility testing (p-value = 0.000, 0.039 and 0.035, respectively) and relatively significant to Amikacin (p-value= 0.068). Also there was significant association between the presences of *hly* gene and amikacin, ciprofloxacin and co-trimoxazole susceptibility testing (p-value = 0.002, 0.002 and 0.041, respectively) and relatively significant to gentamicin (p-value = 0.073) *fimH* gene statistically associated with co-trimoxazole susceptibility testing (p-value = 0.042) (Table 4). The frequency of virulence genes in stool and urine specimens is shown in Table 5, the most frequent multiple genes present in one isolate were pap an *fimH* genes in both urine and stool samples. However, when a single virulence gene frequency was considered, it was found that *fimH* is the most frequent among urine isolates while *aer* is the most frequent among stool isolates.

DISCUSSION

The gene of importance as indicated by the results of the present study, was shown to be *fimH,* which is present

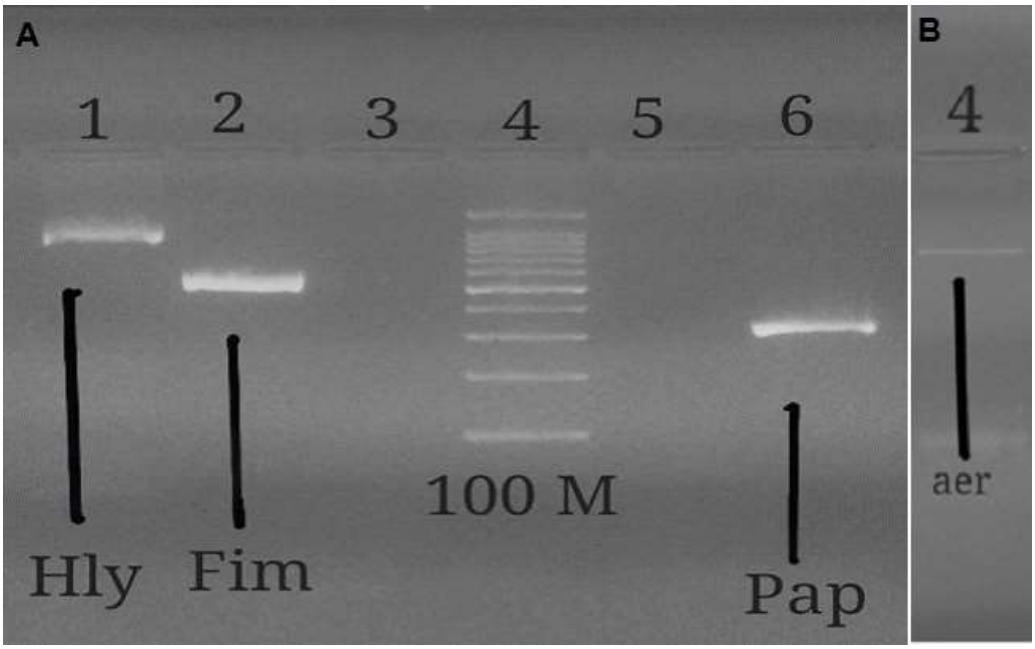

Figure 1. Agarose gel electrophoresis of multiplex PCR product: A; 1= positive *hly* gene, 2: positive *fimH* gene, 3, 5: negative samples, 4: 100 bp ladder, 6: positive *pap* gene, B: positive *aer* gene

Table 4. The association between the presence of UPEC virulence genes and antimicrobial susceptibility testing.

Gene		*Pap*		*fim*		*Sfa*		*aer*		*hly*		Total
Antibiotics		**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	
gentamicin	S	10	45	21	34	9	46	23	32	6	49	55
	R	23	22	20	25	6	39	23	22	11	34	45
	P-value	0.000		0.526		0.673		0.354		0.073		
Amikacin	S	30	66	38	58	14	82	43	53	14	82	96
	R	3	1	3	1	1	3	3	1	3	1	4
	P-value	0.068		0.158		0.568		0.235		0.002		
ciprofloxacin	S	14	43	21	36	5	52	23	34	4	53	57
	R	19	24	20	23	10	33	23	20	13	30	43
	P-value	0.039		0.330		0.045		0.192		0.002		
Co-trimoxazole	S	16	47	21	42	7	56	29	34	7	56	63
	R	17	20	20	17	8	29	17	20	10	27	37
	P-value	0.035		0.042		0.155		0.993		0.041		

+ve: Positive, -ve: negative, S: sensitive; R: resistant.

with high frequency (66%) in urine isolates as compared to the rest of the genes; this may indicate its essential role in *E. coli* causing UTI among Sudanese patients. It is well documented that type 1 pili are important for the invasion and persistence of the UPEC in the urinary bladder after its colonization. Type 1 pili colonization is enhanced by adhesin FimH (Hannan et al., 2012) encoded by fimH gene which recognizes certain α1β3 integrins (Guiton et al., 2012; Eto et al., 2007). It was also confirmed that FimH is important in pathogenesis and that the pathogenicity of a UPEC strain depends greatly on the ability of FimH to switch between conformations and this is also dependent on the different alleles that can be expressed by this gene, affecting FimH conformation

Table 5. Frequency of virulence gene(s) of *E. coli* isolated from urine and stools specimens.

Source of isolate	Number of genes	Number of isolate	*pap*	*fimH*	*sfa*	*aer*	*Hly*
Urine N=44	Four genes N=7	2	+	+	+	+	
		4	+	+	+	-	+
		1	+	-	+	+	+
	Three genes N=11	4	+	+	+	-	-
		1	+	-	+	+	-
		5	+	+	-	-	+
		1	-	+	-	+	+
		4	+	+	-	-	-
	Two genes N=13	1	-	+	+	-	-
		3	-	+	-	+	-
		2	+	-	-	+	-
		1	+	-	-	-	+
		2	-	-	-	+	+
	One gene N=13	9	-	+	-	-	-
		2	-	-	+	-	-
		2	-	-	-	+	-
	Three genes N=1	1	+	+	-	+	-
Stool N=39	Two genes N=11	3	+	+	-	-	-
		4	+	-	-	+	-
		1	+	-	-	-	+
		1	-	+	-	+	-
		2	-	-	-	+	+
	One gene N=27	24	-	-	-	+	-
		3	-	+	-	-	-

+: Positive; -: Negative.

and function (Schwartz et al., 2013). These results agree with published reports, which emphasize the predominance of fimbriae type 1 among the UPEC strains (Jalali et. al., 2015; Tarchouna et al., 2013; Usein et al., 2001). One important virulence factor of *E. coli* causing UTI is fimbriae-mediated adherence. While the role of type 1 fimbriae in virulence is unknown, it is well defined that specific adherence and increased induction of mucosal inflammation are the mechanisms used by P fimbriae to increase the virulence of UPEC (Connell et al., 1996). Significant association (p-value 0.006) was found between *fimH* gene and gender; this association may be due to difference in anatomical structure of urinary tract between male and female (Hickling et al., 2015).

The results confirmed the existence of *aer* gene with more prevalence in diarrheal isolates, this finding totally agreed with Oswald et al. (1991), who found that *aer* gene was positive in 70% of diarrheal samples and Micenková et al. (2014) who found *aer* gene was positive in 68%. High frequency of *aer* gene in diarrheal isolates may be attributed to the deficiency of iron concentration within gastrointestinal tract, while iron is responsible for microbial metabolism. Excretion of siderophores, such as enterobactin is a method by which most *E. coli* can increase access to iron. Aerobactin, salmochelin, and yersiniabactin are three other siderophores linked with pathogenesis, produced by a smaller proportion of isolates (Meyrier, 1999). Prevalence of aerobactin, which confers the ability to bind iron among isolates, was similar to those reported by other investigators in diarrhea isolates. The result show 64% *aer* gene positive in 50 diarrhea isolates and this percentage is near to other researches (Oswald et al., 1991) result of 70% *aer* gene positive and Micenková et al. (2014) result is 68% *aer* gene positive.

The result shows 24/50 (48%) *pap* gene positive in 50 urine isolate and this result is not far from Jalali et al. (2015) who found 46% *pap* positive gene, and Tarchouna et al. (2013), who found 41% *pap* positive gene. Pyelonephritis associated pili (*pap*) play an important role in the pathophysiology of pyelonephritis caused by *E. coli* (Tarchouna et al., 2013). UPEC colonize the bladder by binding urinary tract endothelial cells through utilization of P fimbria that bind D-galactose-D-galactosemoieties on

uroepithelial cells (Todar, 2007).

The result shows 14/50 (28%) *hly* gene positive in 50 urine isolate and this percentage is less than the result of Jalali et al. (2015) who found (47%) *hly* positive gene. There was a clear relation between tissue damage and the presence of hemolysin. Prevalence of these genes differs on the basis of phylogenetics, geographical distribution, and clinical presentation (Oliveira et al., 2011; Blanco et al., 1997). A huge variation in the frequencies of these genes was recorded worldwide (Abe et al., 2008).

When antibiotic resistance was investigated among the virulent isolates, a significant relation was observed since strains carrying the virulence genes were more resistant to several antibiotics. This agrees with a previous study from Iran (Derakhshandeh et al., 2015).

Eighteen isolate (18%) of *E. coli* were negative for UPEC virulence genes and sensitive to all antibiotics used in this study, 12 of them were in diarrheal samples and 6 were in urine samples. These negative isolates may be part of the normal flora that lack these virulent genes or may be due to the possibility of corresponding gene mutations, as negative PCR does not indicate the absence of the corresponding operon while a positive PCR usually confirms the presence of the virulence genes (Tarchouna et al., 2013).

Conclusion

The present study results showed that the UPEC strains isolated in Sudan have a different virulence profile when compared with other studies and it seems that the virulence of UPEC strains depends on the regional geography and climate. However, a recent study in Egypt showed similar distribution of virulence genes (Morsi and Elsaid Tash, 2016). This indicates that some social and environmental factors may contribute in the virulence pattern of UPEC in different communities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abe CM, Salvador FA, Falsetti IN, Vieira MA, Blanco J, Blanco JE, Blanco M, Machado AM, Elias WP, Hernandes RT, Gomes TA (2008). Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic *E. coli.* FEMS Immunol. Med. Microbiol. 52:397-406.

Arisoy M, Aysev D, Ekim M, Özel D, Köse SK, Özsoy ED, Akar N (2005). Detection of virulence factors of *Escherichia coli* from children by multiplex PCR. Int. J. Clin. Prac. 60 (2):170-173.

Bahalo S, Tajbakhsh E, Tajbakhsh S, Momeni M, Tajbakhsh F (2013). Detection of some virulence factors of *Escherichia coli* isolated from urinary tract infection isolated of children in Shahrekord Iran by Multiplex PCR. Middle-East J. Sci. Res. 14(1):29-32.

Bahrani-Mougeot F, Gunther NW, Donnenberg MS, Mobley HLT (2002). "Uropathogenic *Escherichia coli*." In *Escherichia coli:* Virulence Mechanisms of a Versatile Pathogen. MS Donnenberg (ed). London: Academic Press. pp. 239-268.

Blanco M, Blanco JE, Rodrıguez E, Abalia I, Alonso MP, Blanco J (1997). Detection of virulence genes in uropathogenic *Escherichia coli* by PCR comparison with results obtained using phenotypic methods. J. Clin. Micro. 31:37-43.

Connell I, Agace W, Klemm P, Schembri M, Mărild S and Svanborg C (1996). Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proc. Nat. Acad. Sci. 93(18):9827-9832.

Czaja CA, Stamm WE, Stapleton AE, Roberts PL, Hawn TR, Scholes D, Samadpour M, Hultgren SJ, Hooton TM (2009). Prospective cohort study of microbial and inflammatory events immediately preceding *Escherichia coli* recurrent urinary tract infection in women. J. Infect. Dis. 200:528-536.

Derakhshandeh A1, Firouzi R, Motamedifar M, Arabshahi S, Novinrooz A, Boroojeni AM, Bahadori M, Heidari S (2015). Virulence Characteristics and Antibiotic Resistance Patterns among Various Phylogenetic Groups of Uropathogenic *Escherichia coli* Isolates. Jpn. J. Infect. Dis. 68(5):428-431.

Dobrindt U, Blum-Oehler G, Nagy G, Schneider G, Johann A, Gottschalk G, Hacker J (2002). "Genetic structure and distribution of four pathogenicity islands (PAI I536 to PAI IV536) of uropathogenic *Escherichia coli* strain 536." Infect. Immunity 70:6365-6372.

Eto DS, Jones TA, Sundsbak JL, Mulvey MA (2007). Integrin-mediated host cell invasion by type 1-piliated uropathogenic *Escherichia coli*. PLoS Pathog; 3:e100

Guiton PS, Cusumano CK, Kline KA, Dodson KW, Han Z, Janetka JW, Henderson JP, Caparon MG, Hultgren SJ (2012). Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice. Antimicrob Agents Chemother. 56:4738-4745.

Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA, Hultgren SJ (2012). Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. FEMS Microbiol. Rev. 36:616-648.

Hickling DR, Sun TT, Wu XR (2015). Anatomy and Physiology of the Urinary Tract: Relation to Host Defense and Microbial Infection. Microbiology spectrum 3(4): doi: 10.1128/microbiolspec.UTI-0016-2012.

IBM SPSS Statistics for Windows, Version 21.0. (2012). Armonk, NY: IBM Corp, Released. www.ibm.com/software/analytics/spss/ IBM Corp.

Jalali HR, Pourbakhsh A, Fallah F, Eslami G (2015). Genotyping of Virulence Factors of Uropathogenic Escherichia coli by PCR. Novelty in Biomed. 3(4):177-181.

Johnson JR (2002) "Evolution of pathogenic *Escherichia coli*." In *Escherichia coli:* Virulence Mechanisms of a Versatile Pathogen. M. S. Donnenberg(ed). London: Academic Press, pp. 55-77.

Johnson JR (1991). Virulence factors in *Escherichia coli* urinary tract infection. Clin Microbiol Rev. 4:80-128.

Mabbett AN, Ulett GC, Watts RE, Tree JJ, Totsika M, Cheryl-lynn YO, Wood JM, Monaghan W, Looke DF, Nimmo GR, Svanborg C (2009). Virulence properties of asymptomatic bacteriuria *Escherichia coli* . Int. J. Med. Microbiol. 299:53-63.

Meyrier A (1999). Urinary Tract Infection. In: Schrier RW, Cohen AH, Glassock RJ, Grünfeld JP. Atlas of diseases of the kidney (PDF). 2. *Oxford: Blackwell Sci*. ISBN 0-632-04387-3.

Micenková L, Štaudová B, Bosák J, Mikalová L, Littnerová S, Vrba M, Ševčíková A, Woznicová V, Šmajs D (2014). Bacteriocin-encoding genes and ExPEC virulence determinants are associated in human fecal *Escherichia coli* strains. BMC Microbial. 14(1):109.

Middendorf B, Hochhut B, Leipold K, Dobrindt U, Blum-Oehler G, Hacker J (2004) "Instability of pathogenicity islands in uropathogenic *Escherichia coli* 536." J. Bacteriol.186:3086-3096.

Mobley HL, Green DM, Trifillis AL, Johnson DE, Chippendale GR, Lockatell, CV, Jones BD dan Warren JW (1990). Pyelonephritogenic *Escherichia coli* and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. Infect. Immunol. 58:1281.

Morsi SS, Tash RM (2016). Virulence Determinants among Extended-Spectrum B- Lactamases producers of Uropathogenic *Escherichia coli* isolates In Zagagig University Hospitals, Egypt, Egyptian J. Med. Microbiol. 25(2):101-108.

Obata-Yasuoka M, Ba-Thein W, Tsukamoto T, Yoshikawa H, Hayashi H (2002). Vaginal *Escherichia coli* share common virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*. Microbiology 148:2745-2752.

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. Genet. Mol. Res. 10(4):4114-25.

Oswald E, de Rycke J, Lintermans P, van Muylem K, Mainil J, Daube G, Pohl P (1991). Virulence factors associated with cytotoxic necrotizing factor type two in bovine diarrheic and septicemic strains of *Escherichia coli*. J. Clin Microbiol. 29:2522-2527.

Ribeiro TIBA M, Yano T, Da Silva LEITE D (2008). Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. Rev Inst. Med. Trop. S Paulo. 50(5):255-60.

Salyers AA, Whitt DD (2002). Bacterial pathogenesis: a molecular approach. Washington DC: ASM Press.

Schneider G, Dobrindt U, Brüggemann H, Nagy G, Janke B, Blum-Oehler G, Buchrieser C, Gottschalk G, Emödy L, Hacker J (2004) "The pathogenicity island-associated K15 capsule determinant exhibits a novel genetic structure and correlates with virulence in uropathogenic *Escherichia coli* strain 536. Infect. Immun. 72:5993-6001.

Scholes D, Hooton TM, Roberts PL, Gupta K, Stapleton AE, Stamm WE (2005). Risk factors associated with acute pyelonephritis in healthy women. Ann. Int. Med.142:20-27.

Schwartz DJ, Kalas V, Pinkner JS, Chen SL, Spaulding CN, Dodson KW, Hultgren SJ (2013). Positively selected fimH residues enhance virulence during urinary tract infection by altering fimH conformation. Proc Natl. Acad. Sci. USA.; 110:15530-15537.

Tarchouna M, Ferjani A, Selma WB, Boukadida J (2013). Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. Int. J. Infect. Dis.17:450-3.

Todar K (2007). Pathogenic *E. coli*, in *Online Textbook of Bacteriology*, University of Wisconsin-Department of Bacteriology.[homepage on internet] cited on -11-30. Available from http://www.textbookofbacreiology.net/.

Usein CR, Damian M, Tatu-Chitoiu D, Capusa C, Fagaras R, Tudorache D, Nica M, Bouguénec C (2001). Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. J. Cell. Mol. Med. 5(3):303-310.

Welch RA, Burland V, Plunkett GI, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D (. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proceedings of the National Academy of Sciences. 99(26):17020-17024.

Wullt B (2003). "The role of P fimbriae for *Escherichia coli* establishment and mucosal inflammation in the human urinary tract." Int. J. Antimicrob. Agents 21:605-621.

Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O (1995). Detection of urovirulence factors in *Escherichia coli* by multiplex PCR. FEMS Immuno. Med. Microbiol. 12:85-90.