JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 425-428

A Study on Adherence of Multiple Antibiotic Resistant Strains of *Escherichia coli* to Uroepithelial Cells

Mohammed A. Ibrahim[1]* and Ahmed K. Ibraheem[2]

[1]Royal Scientific Society, Environmental Research Center, Amman, Jordan.
[2]Department of Biotechnology, Al-Nahrain University, Baghdad, Iraq.

(Received: 05 May 2009; accepted: 26 June 2009)

200 urine samples were collected from patients expected to have urinary tract infections. *E. coli* was identified in 41 positive cultures which showed bacterial growth. Sensitivity tests indicated that Ciprofloxacin, Amikacin, and Naldixic acid were the most effective antibiotics and resistance percentage of E. coli isolates to these antibiotics were 5%, 5%, and 9.8% respectively; while Ampicillin, Cephalexin, and Erythromycin were not effective. Six *E. coli* isolates designated (EC5, EC16, EC30, EC31, EC35, and EC41) with multiple antibiotic resistance were found to show variation in respect of adherence to uroepithelial cells. Isolate EC5 showed the highest ability of adherence with the mean number of adhering bacteria equivalent to 34 bacteria per uroepithelial cell. Plasmid curing experiments showed that isolates (EC16, EC30, and EC35) lost their antibiotic resistance determinants of Cotrimoxazole, Gentamicin, Naldixic acid, Tetracycline, and Ciprofloxacin, indicating that resistance to these antibiotics are under plasmid control, whereas Ampicillin, Cephalexin, Erythromycin, and Cefotaxime resistant determinants were not affected as well as the ability for adherence to uroepithelial cells.

Key words: *E. coli*, Antibiotic resistance, Adherence, Uroepithelial cells, Plasmid.

Bacterial adherence to host cells is a crucial step in the initiation of various infections, thus, investigation of attachment of uropathogenic *E. coli* (UPEC) to specific sites on uroepithelial cell is of a great of importance in studying various aspects of pathogenesis of UPEC. Uropathogenic *E. coli* strains possess an array of adhesins including, type 1, P, S, and G fimbriae, and non fimbriae adhesins (NFAs)[1,2]. It had been reported that adherence factors facilitate the colonization and persistence in the urinary tract[3]. The adherence of bacteria to uroepithelial cells (UECs) assists bacteria to resist the mechanical wash by the flow of urine, which enables them to persist, colonize and invade the tissue[4]. Eden in 19805 found that most of the *E.coli* strains tested attached to both sequamous and transitional epithelial cells. Hardy, 1986 indicated that plasmids are involved in conferring bacterial virulence and can make their host more pathogenic, for example colonization factor antigens CFAs (adhesins) which are plasmid coded, enable the Enterotoxigenic *E.coli* strains to adhere intestinal cells. Plasmid-associated resistance genes of *E. coli* have been discovered for the majority of known antimicrobials,

* To whom all correspondence should be addressed.
E-mail: obaide@rss.gov.jo; prof_mibrahim@yahoo.com

including the ß-lactams, quinolones, tetracyclines, chloramphenicol and trimethoprime[7,8,9,10], thus, *E. coli* is considered as candidate species in which new multi-resistance plasmids may be evolved. In this study an investigation was carried out to ascertain the possible effect of multiple antibiotics resistance on adherence ability of uropathogenic *E. coli* to uroepithelial cells.

MATERIAL AND METHODS

Collection of urine samples

Midstream urine samples were collected in sterile cups from patients of outpatient private urology clinic and central public health laboratory in Baghdad.

Bacterial isolation and characterization

Loopful of undiluted urine samples were spread on blood agar and MacConkey Agar plates, incubated overnight at 37° C, single colonies were observed for their lactose fermenting ability, then the colonies which showed positive reaction were transferred to new MacConkey agar plate for further purification by dilution streaking to obtain single isolated colonies used for further biochemical ,physiological and API 20 E tests.

Antibiotic sensitivity

The sensitivity tests were determined according to the National Committee for Laboratory Standard.

Table 1. Percentages of antibiotic resistance in 41 isolates of *E. coli* isolated from UTI samples

Antibiotics	No. of resistant isolates	Resistance %
Ampicillin(AM)	41	100
Amoxicillin + clavulanic acid(AMC)	36	88
Cephalexin(CL)	41	100
Cefotaxime(CTX)	34	83
Streptomycin(S)	34	83
Gentamycin(CN)	21	51
Amikacin(AK)	2	5
Erythromycin(E)	41	100
Tetracycline(TE)	15	36.6
Naldixic acid(NA)	4	9.8
Ciprofloxacin(CIP)	2	5
Chloramphenicol(C)	7	17
Nitrofuration(F)	24	58.5
Trimethoprim + slfamethoaxazole(SXT)	26	63

Adhesion test

Six isolates of *E. coli* were selected according to their multiple antibiotics resistance, each isolate was cultured in 10 ml nutrient broth, incubated overnight at 37° C and prepared for adhesion test according to the procedure reported by Iwahi *et al.*, 1982 [11].

Plasmid curing

Acridine orange (AO) was used as curing agent for plasmid DNA, the experiment was done as described by Miller, 1972 [12]. Plasmids DNA

Table 2. The adherence of *E. coli* (EC series) to uroepithelial cells

Isolate No. of *E.coli*	Multiplicity of antibiotic resistance	In phosphate buffer saline (PBS) No. of adherent *E.coli* to no. of uroepithelial cells				Mean no. of adherent *E.coli*/cell
		0	1-5	6-2	>20	
5	8	0	0	3	17	34
16	7	4	3	10	3	18
30	9	0	1	1	18	33
31	11	5	2	11	2	16.4
35	13	2	2	4	12	29.9
41	7	0	0	3	17	29
Final mean number of adherent *E.coli* / uroepithelial cell for all *E.coli* isolates						26.6

were isolated by alkaline lysis method and bands were detected on agarose gel electrophoresis[13].

RESULTS AND DISCUSSION

Two hundred midstream urine samples were collected from patients suffering from symptoms referred as urinary tract infection. 123 samples gave positive bacterial growth on MacConkey and blood agar plates, 41 positive cultures were identified as *E. coli* (33.3%). 66% of *E. coli* cultures were from females while 34% of *E. coli* isolates were identified in male samples. Antibiotic sensitivity tests against 14 antibiotics showed that ciprofloxacin, amikacin, and Naldixic acid were the most effective antibiotics and their resistance percentage were 5%, 5%, and 9.8% respectively. While ampicillin, cephalexin, and erythromycin were not effective and their resistance percentage were 100% (Table 1). These results might suggest the existence of evolutionary process of antibiotic resistance which builds up gradually in *E. coli* in specific pattern[14].

The results in table 2 demonstrate that *E. coli* (EC 5) which is resistant to eight antibiotics gave the highest mean number of adhering bacterial cells to uroepithelium cell (34 bacteria per uroepithelium cell). Whereas *E. coli* (EC 31) which is resistant to 11 antibiotics showed the lowest mean number of adherent bacterial cells to uroepithelium cell (16 bacteria/ cell), other tested *E. coli* isolates gave adherence values which were in the range between 18 - 33 bacteria /cell.

This observation confirm earlier invistigations which correlate the ability of *E.coli* to adhere to uroepithelial cells and pathogenesis of UTI[3,15]. Moreover, these results need further work to ascertain possible correlation between degree of multiplicity of antibiotics resistance, adherence ability and plasmids contents of tested bacteria.

Table 3 shows the results of plasmid curing experiments after acridine orange treatment, streptomycin and SXT (Trimethoprim +Sulfamethaxzole) resistance determinants were

Table 3. Antibiotic resistance of *E.coli* isolates (EC series) before and after acridine orangr (AO) treatment

Isolate No. of *E.coli*	Pre-curing		Post-curing	
	Resistance patterns	Plasmid profiles	Resistance patterns	Plasmid profiles
16	AMP, CL, E, CTX, AMC, S, SXT	two bands,	AMP, CL, E, CTX, AMC	No plasmid bands
30	AMP, CL, E, CTX, AMC, S, CN, TE, AK	two bands	AMP, CL, E, CTX, AMC, S	No plasmid Bands
31	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, C	three bands	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, C	three bands
35	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, N.A, CIP	four bands	AMP, CL, E, CTX	No plasmid bands

Table 4. Adherence of *E. coli* (EC series) to Uroepithelial cells before and after AO treatment.

Isolate no. of *E.coli*	In phosphate buffer saline (PBS)	
	Mean no. of adherent *E. coli* / cell before AO treatment	Mean no. of adherent *E.coli* / cell after AO treatment
16	19	18
30	32	32
35	29	29.2

lost in isolate (EC 16) whereas, gentamycin, tetracycline and amikacin resistance determinants were lost in isolate (EC 31), on the other hand

AMC(amoxicillin +clavulanic acid), streptomycin, gentamycin, tetracycline, SXT, Nitrofuration, Ciprofloxacin resistance determinants were lost in isolates (EC 35) after treatment of *E. coli* with acridine orange. Loss of resistance was accompanied by disappearing of the plasmid bands in the three isolates. Table(4) shows the adherence ability of plasmid cured E. coli, the obtained results indicated that removal of plasmids from bacterial cells did not affect adherence, thus, suggesting that adherence ability of *E. coli* isolates to uroepithelial cells were not affected by R plasmids contents of *E. coli* cells.

CONCLUSIONS

High percentage of *E. coli* isolates were resistant to ampicillin, erythromcin, cephalexin, amoxicillin/clavulanic acid combination, while the most effective antibiotics against UPEC were amikacin and ciprofloxacin.

Acridine orange showed a powerful activity as curing agent for elimination of plasmid(s) responsible for antibiotics resistance in *E. coli*. Moreover the study showed no correlation between adhesion ability of UPEC and multiplicity of antibiotics resistance.

ACKNOWLEDGMENTS

The authors are grateful and express their appreciation to Royal Scientific Society and to Department of Biotechnology, Al-Nahrain University for facilitating and supporting this research.

REFERENCES

1. Maning, S. D.; Zhang, L.; Foxman, B.; Spindler, A.; Tallman, P. and Marrs, C. F.. Prevalence of known P- fimbrial G alleles in *E.coli* and identification of a new adhesion class. *J. Clin. And Diagno. Lab. Immun.,* 2001; **8**(3):637-640.
2. Tolkoff-Rubin, N. E.; Cotran, R. S. and Rubin, R. H.. Urinary tract infection, pyelonephritis, and reflux nephropathy. P.1513. In: Brenner and Rector's The Kidney, Brenner, B. M. (seventh Ed), 2004; Vol. 2 .Ch.31. Saunders. USA.
3. Zhang, L. ; Foxman, B.; Tallman, P.; Claderd, E.; Le Bouguenec, C. and Marrs, C. F.. Distribution of drb genes coding for Dr. binding adhesions among uropathogenic and fecal *E.coli* isolates and identification of new subtypes. *J. Infe. And Immun*., 1997; **65**(6): 2011 - 2018.
4. Hagberg, L.;Jodal, U.; Korhonen, T. K.; Janson, G. L.; Lindberg, U. and Eden, C. S.. Adhesion, heamagglutination, and virulence of *E.coli* causing urinary tract infections. *J. Infect. And Immun*., 1981; **31:** 564-570.
5. Eden, C. S. Larsson, P. and Lomberg, H.. Attachment of *P. mirabilis* to human urinary sediment epithelial cells in vitro is different from that of *E. coli. Infec. and Immun.* 1980; **27**(3): 804-807.
6. Hardy, K. Bacterial plasmids. (2nd Ed.). American society for microbiology. 1986; USA.
7. Chambers, H. F. Chemotherapeutic drugs. In: Basic and Clinical Pharmacology. Katzung, B. G. (eighth Ed.). 2001; Lange Medical Books / McGraw-Hill. USA.
8. Forbes, B. A.; Sahm, D. F.; Weissfeld, A. S.."Baily and scott's diagnostic microbiology". 1991; (10th Ed).Mosby Inc. Missouri.
9. Nester, E. W.; Anderson, D. G.; Roberts, C. J.; Pearsall, N. N.; Nester, M. T. " Microbiology A Human Prespective". (third Ed.). 2001; McGraw-Hill, Higher Education, NY.
10. Sotto, A. ; Boever, C.M.; Fabbr-peray, P.; Gouby, A.; Sirot, D. and Jourdan, J.(). Risk factors for antibiotic-resistance *E.coli* isolated from hospitalized patients with UTIs: a propective study. *J. Clin. Microbiol.,* 2001; **39**(2):438-444.
11. Iwahi, T.; Abe, Y. and Tsuchiya, K. Virulence of *E.coli* in ascending urinary tract infection in mice. *J. Med. Microbio.,* 1982; **15:** 303-316.
12. Miller, J. H.. Experiments in molecular genetics. Cold spring harbor laboratory, 1972; New York.
13. Sambrook, J.; Fritgah, E. and Maniatis, T.. Molecular cloning: a laboratory manual. (Second Ed.). 1989; Cold spring, Herbour lab. New York.
14. Sherley, M.; Gordon, D. and Collignon, P.J. Evolution of multi-resistance plasmids in Ausralian clinical isolates of *E.coli. Microbiology,* 2004; **150:** 1539- 1546.
15. Ziegler, T.; Jacobsohn, R. and Funfstuck, R. Correlation between blood group phenotype and virulence properties of *E.coli* in patients with chronic urinary tract infection. *J. Antimicrob. Agents*, 2004; **24S:** S70-S75.