The Location of Aerobactin Determinants of Uropathogenic *E. coli* in Association with Hemolysin Production Antibiotic Resistance and Patient Characteristics.

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ABSTRACT:

BACKGROUND:

Aerobactin, Hemolysin and the resistance to some antibiotics axe important Features of Uropathogenic *E. coli* (UPEC). The characteristics of patients from which we isolate the UPEC have associate with these Features and their determinants location.

AIM:

We determined the phenotypic expression and gene location of Aerobactin and phenotypic expression of Hemolysin among 60 UPEC isolates. We correlated the presence of Aerobactin system with antibacterial agents resistance. The expression of Hemolysin and the characteristics of patients.

METHODS:

Two methods of plasmid curin, sodium dodecyl sulfate and elevated temperature plasmid curing, are used then plasmid extraction and agarose gel electrophoresis are performed to determine the location of Aerobactin determinants and the size of Aerobactin plasmids, as well as the location of the determinants of some antibiotics resistance which were ampicillin (Am), Tetracycline (Tc), gentamicin (GM), Chloramphenicol (C) and co- trimazole (Co).

RESULTS:

Aerobactin and hemolysin expression among UPEC isolates were 93.3% and 35% respectively. The isolates of non compromised patients produce statistically higher rate of expressed hemolysin (90.5% , $p<0.01$). Plasmid- borne Aerobactin was absent in UPEC isolates of non compromised patients (89.5%, $p<0.01$). On the contrary compromised patient isolates express plasmid Aerobactin of 59.5% ($p<0.02$). We also found that Aerobactin determinants are located on a plasmid in compromised patient isolates and associated with the absence of chromosomal Hemolysin production (82.9%, $p<0.01$). Yet, The chromosomal aerobactin is associated with hemolysin production (61.9%, $p<0.02$). Furthermore, UPEC isolates of compromised patients carry relatively large plasmid of Aerobactin (85.7%. $p<0.05$) and these large plasmids carry either chloramphenicol (83.3%.$p<0.02$) or gentamicin determinants (100%. $P<0.01$) but not co- trimazole, tetracycline or ampicillin.

CONCLUSION:

The isolates of non compromised patients carry chromosomal Aerobactin and hemolysin. While the isolates of compromised patients express plasmid Aerobactin and do not express chromosomal hemolysin. Aeobactin plasmids are relatively large plasmids and carry either chloramphenicol or gentamicin resistance determinants.

KEY WORDS: Uropathogenic *E .coli*. Aerobactin. Hemolysin. Antibiotics. Compromised . Non compromised.

INTRODUCTION:

Aerobactin is a derivative of hydroxamic acid. Produced by enteric bacteria like *E. coli* [1,2]. *Shigella* spp and *Klebsiella* spp.[3]The determinants that encode the production of Aerobactin are located either on a plasmid or on the chromosome of uropathogenic *E. coli*, UPEC [1.4].

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The determinants of some antibiotics resistance like chloramphenicol, gentamicin, co- trimazole, ampicilin and tetracycline are carried on the Aerobactin plasmids [5.6]. Such plasmids are usually relatively large plasmids called P Col V plasmids.[7] Hemolysins (Hly) are group of phospholipases, produced by pathogenic *E.coli* strains and had been demonstrated as chromosomal occurance virulence factor (VF). The present study determine the prevalence extent of expression, chromosomal versus plasmid location of the genetic determinants for Aerobactin and to characterize interrelationship between hemolysin

and aerobactin. In addition, we sought to determine the presence and expression of aerobactin determinants among *E. coli* strains from patients with urinary tract infections (UTIs) who have compromising urological and medical conditions .We also investigated the association between the presence of the determinant of aerobactin and resistance to some antibiotics (ABs).

PATIETS, MATERIALS AND METHODS:

A total of 321 midstream urine samples were collected from hospitalized and non- hospitalized patients (of both sexes and different ages), complaining of UTI symptoms attending AL-Kadhymia Teaching Hospital- Baghdad. 238 patients had UTI symptoms without predisposing factors (PF_s) of infection and about 81 patients had UTI symptoms with PF_s (diabetes, n=4; urinary catheterization, n=4; urinary stones, n=49; prostate enlargement, n=4; leukemia, n=16; bladder CA, n=4). For isolation and identification of uropathogens, blood and MacConkey agars (Oxoid- UK) were used and a set of biochemical tests. Lambda phage DNA (Boehringer- Germany) serves as molecular marker during electrophoresis. A method of Dillon *et al*, 1985[8] and Ausubal *et al*, 1987[9] was used for plasmid DNA extraction. Minimum growth medium (M9) supplemented with 2,2 dipyridyl (BDH-UK), a chelating agent, was prepared according to Johnson *et al* (1988)[1] method. The detection of Hly production was performed using 5% blood agar plates. Disc diffusion test for AB_s susceptibility was applied according to Treagan and Pullian (1982)[10] and Vandepitte *et al* (1991)[11]. Elevated temperature at 43°c[12] and 10% sodium dodecyl sulfate (SDS)[13] methods were used for plasmid curing. The data were analyzed by chi- square test which was applied for the comparison among different groups when the enumerative data were qualitative.[14]

REULTS:

1.patients and culture results:

Out of 238 urine samples that obtained from patients suffering from the symptoms of UTIs without PFs, only 49 gave positive culture results. While urine samples that were obtained from patients who had symptoms of UTIs in association with PFs and showed positive culture results were 75. *E. coli* isolates were the most predominant bacteria, 60 isolates (48.4%) among uropathogens, 25 isolates are obtained from compromised patients (with PFs). While the other 35 isolates were from samples of non compromised patients.

2.Aerobactin production:

Out of 60 UPEC isolates that were tested for Aerobactin production, 56 (93.3%) isolates gave positive results.

3.plasmid curing:

All of 56 UPEC isolates were submitted to two methods of plasmid curing, elevated temperature method (at 43 °C) and 10% SDS plasmid curing method. Curing by elevated temperature depends on the differences in the replication rate between the plasmid and chromosomal DNA at 43°C.[15] While curing with 10% SDS relys on the principle of cell membrane disruption.[13] *E. coli* isolates that fail to grow on M9 supplemented media with 2,2 dipyridyl after curing, may carry Aerobactin determinants on a plasmid, on other hand. *E. coli* isolate that can grow on such media after its submission to the curing procedure, probably carring these determinants on its chromosome. Out of these 56 Aerobactin expressed isolates, 37 (66%) lose the aerobactin production phenotype, 26 of them were lost by both curing methods, three by SDS and eight by elevated temperature method (Table-1 and Figure 1).

Table1: The loss of aerobactin production feature of uropathogenic *E. coli* after plasmid curing with elevated temperature and sodium dodecyl sulfate (SDS) methods.

Loss of aerobactin production feature after plasmid curing with	No. of the isolates n=37
Elevated temperature	8
SDS only	3
Both of them	26

As shown in table 2, *E.coli* isolates of non compromised patients tend to preserve such feature after curing, and plasmid- borne aerobactin is absent in the isolates of non compromised patients (89.5%, $p<0.01$). On the contrary, the isolates of compromised patients express plasmid aerobactin (59.5%, $p<0.02$).

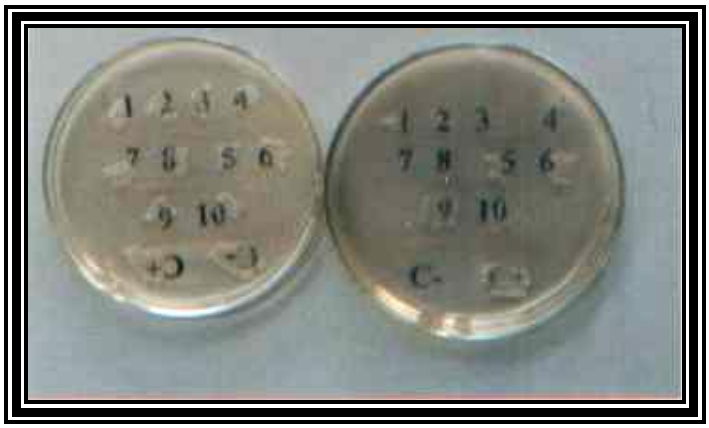

Figure 1: The loss of the aerobactin production feature of uropathogenic *E. coli* after plasmid curing with elevated temperature. The plate on the right contain M9 media supplemented with 2,2 dipyridyl whereas, the one on the left contains M9 media without 2,2 dipyridyl. C- = control negative
C + = control positive 1-10 = The streak of well- isolated colonies obtained from single colony submitted to the elevated temperature plasmid curing. Colonies number 2,3,4,7 and 8 cannot grow on medium containing a chelating agent due to the loss of aerobactin plasmid.

Table 2: plasmid- borne aerobactin in *E. coli* isolates of compromised and non compromised patients.

Patient groups	Plasmid aerobactin	
	Present n=37 (66%)	Absent n=19 (34%)
Non- compromised	15 (40.5%)	17 (89.5%)[a]**
Compromised	22 (59.5%) [b]*	2 (10.5%)

χ^2 test : [a] between patient groups : ** P < 0.01
[b] Within the group : * P < 0.02.

4. Hemolysin production: In table 3 only 35% of UPEC were Hemolysin, and were associated with the isolates of non compromised patients, (90.5%, p< 0.01). On the other hand, table 4 reveals that these isolates were associated with chromosomal aerobactin expression (61.9%, p< 0.02), and tend to be absent in the isolates that express plasmid aerobactin (82. 9%, p< 0. 01).

Table 3: Expressed hemolysin in uropathogenic *E. coli* isolates of compromised and non compromised patients.

Patient groups n=60	Hemolysin	
	Present n=21 (35%)	Absent n=39 (65%)
Non compromised	19 (90.5%)**	16 (41%)
Compromised	2 (9.5%)	23(59%)

Between the groups x^2 test: ** p< 0.01.

Table4: The association between the location of aerobactin determinant and the hemolysin expression feature in uropathogenic *E. coli*.

The location of aerobactin determinant	Hemolysin	
	Present n=21 (37.5%)	Absent n=35 (62.5%)
Chromosomal aerobactin	13 (61.9%)a*	6 (17.1%)
Plasmid aerobactin	8 (38.1%)	29 (82.9%) b**

χ^2 test: [a] between hemolysin expression and chromosomal aerobactin : * P < 0.02
[b] Plasmid aerobactin Vs hemolysin expression = ** P < 0.01.

5. The loss of antibiotics resistance feature: Antibiotic susceptibility to the co- trimazole (CO), tetracycline (Tc) gentamicin (GM), chloramphenicol (C) and ampicillim (Am) wad performed before and after plasmid curing for all of the 37 isolates that showed loss for aerobactin feature after plasmid curing, table 5 shows the loss of resistance to the five antibiotics in association with failure of aerobactin production and patient characteristics after plasmid curing.

Table 5: The association between patient characteristics and the loss of resistance to some antibiotics in association with failure of aerobactin production after plasmid curing.

Patients characteristics	Co-trimazole Resistance		Chloramphenicol Resistance		Ampicillin Resistance		Gentamicin Resistance		Tetracycline Resistance	
	L n=8 (23.5 %)	N n=26 (76.5 %)	L n=21 (91.3 %)	N n=2 (8.7 %)	L n=2 (5.7 %)	N n=33 (94.3 %)	L n=19 (79 %)	N n=5 (21 %)	L n=5 (19.2 %)	N n=21 (80.8 %)
Compromised	6 (75%)	14 (53.8 %)	17 (80.9 %)	1 (50 %)	1 (50 %)	20 (60.6 %)	14 (73.7%)	1 (20 %)	4 (80%)	14 (66.7 %)
Non-compromised	2 (25%)	12 (46.2 %)	4 (19.1 %)	1 (50 %)	1 (50 %)	13 (39.4 %)	5 (26.3%)	4 (80 %)	1 (20%)	7 (33.3 %)

L= The loss of the antibiotic resistance feature following aerobactin loss by plasmid curing .
N=No change in antibiotic resistance feature after curing of aerobactin plasmid.

6- Aerobactin plasmids: 26 *E. coli* isolates that lost the aerobactin production feature after plasmid curing with both methods of curing are submitted to the plasmid extraction and agarose gel electrophoresis before and after curing to screen the loss of the plasmids.21 isolates show the loss of just one plasmid in both cured extracts (table -6). The first or second band, from two to three bands of these isolates, the rest five loss two bands other than first or second bands. Figure 2 shows this fact, the first isolate (lane 2,3 and 4) show the loss of the second band in both elevated temperature (lane 3) and SDS cured extract (lane 4), the third isolate (lane 8,9 and 10) also lose the second which has the molecular size less than lambda phage DNA (48 kb). The second isolate (lane 5,6 and 7) lose the second band which has approximate molecular size of lambda phage DNA. The failure of aerobactin production after curing with both SDS and elevated temperature phenotypically is accompanied with the loss of just one large plasmid in both cured extracts and that mean that the lost plasmid is the aerobactin plasmid. Table 6 shows that the isolates of compromised patients tend to carry relatively large plasmid (85.7%, $p< 0.05$), as compare with those of non compromised (14. 3%).

Table 6: The size of aerobactin plasmid of uropathogenic *E. coli* isolates of compromised and non- compromised patients.

Patient groups	The aerobactin plasmid	
	Large n=21 (80.8%)	Small n=5 (19.2%)
Compromised	18 (85.7%)*	2 (40%)
Non compromised	3 (14.3%)	3 (60%)

Between groups : χ^2 test : * $P < 0.05$

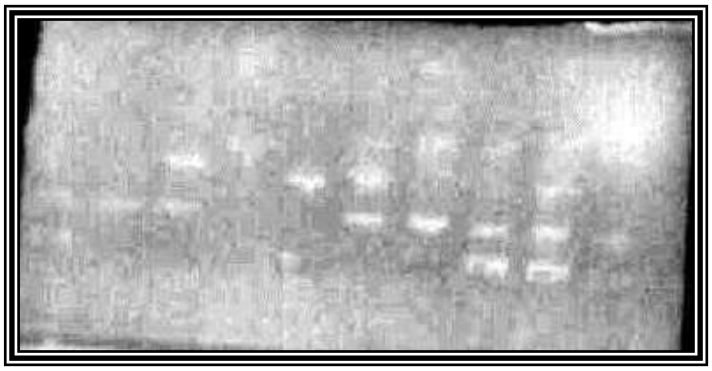

Figure 2: Agarose gel electrophoreticogram of the plasmids isolated from uropathogenic *E coli* (UPEC). Plasmids isolated from three isolates of compromised patients showing . **Lane 1:** Lambda phage DNA (48 kb) as a molecular marker marken. Lane 2,5 and 8, are the plasmid profile of three UPEC isolates before curing.Lane 3,6 and 9. plasmid cured of the previous isolates using temperature. of 43 ^{o}C. Lane 4,7 and 10 plasmid cured by dodecyl sulfate.

7-The plasmids of aerobactin and some antibiotic resistance: It was previously reported that aerobactin plasmid carry the determinants of some antibiotic resistance like C, Tc, Am, Co and GM[(6)]. Of these ABs only C (56.8%) and GM (51.4%) resistance loss were phenotypically associated with the loss of aerobactin (p< 0.05), but not with Co, Tc and Am (Table 7).

Table 7: The pattern of loss of some antibiotics (ABs) resistance following the loss of aerobactin production feature after plasmid curing with sodium dodecyl sulfate (SDS) and elevated temperature.

Antibiotics	Loss of antibiotic resistance following plasmid curing with						Total aerobactin cured isolate n=37	
	SDS only		Temperature only		SDS&temperature			
	No.	%	No.	%	No.	%	No.	%
Co-trimazole	1	12.5	0	0	7	87.5	8	21.60
Chloramphenicol	1	4.8	6	28.6	14	66.6	21	56.8*
Tetracycline	1	20	0	0	4	80	5	13.5
Ampicillin	2	100	0	0	0	0	2	5.4
Gentamicin	5	26.3	6	31.6	8	42.1	19	51.4*

X^2 test: The loss of resistance and aerobactin production feature VS the persistence * p< 0.05.

In table 8, the plasmids that carry the determinants of aerobactin production and chloramphenicol resistance are relatively large plasmids (85.7%) and associated with the isolates of compromised patients (83.3%, p< 0.02), Table 9 shows, that the plasmids that carry the aerobactin determinants accompanied with the determinants of GM resistance, were large and were obtained from compromised patients (100%, p< 0.01)

Table 8: The association between the size of the plasmid of aerobactin and chlormphenicol resistance with patient characteristics.

Patient groups (or characteristics)	Chloramphenicol resistance and aerobactin plasmid	
	Large plasmid n=12 (85.7%)	Small plasmid n=2 (14.3%)
Compromised	10 (83.3%)*	0 (0%)
Non compromised	2 (16.7%)	2 (100%)

Between groups X^2 test:* p< 0.02.

Table 9: the association between the size of the plasmid of aerobactin and gentamicin (GM) resistance with patient characteristics.

Patient characteristics	GM resistance and aerobactin plasmid Large n=7 (87.5%)	small n=1 (12.5%)
Compromised	7 (100%)**	0 (0%)
Non- compromised	0 (0%)	1 (100%)

Between groups X^2 test: ** p< 0.01.

DISCUSSION:

Aerobactin is a bacterial iron sequestration and transport system which enables *E. coli* to grow in iron- poor environments such as dilute urine. Iron is a vital trace element for bacterial growth, the functions of iron in the cells are to activate the electron transport systems,[3]and enzymes like ribonucleotide reductase, catalase, oxidases, superoxide dismutase as well as it enters in the composition of sulfer proteins.[16] Due to these critical functions the aerobactin production and utilization by UPEC reported in our study were high. The presence of direct repeats at both ends of aerobactin genes indicate that the transposition events are responsible for the plasmid and chromosomal location of aerobactin genes.[17] In the present study, plasmid aerobactin associated with the isolates of compromised patients, and the isolates of non compromised patients tend to preserve the feature of aerobactin production after plasmid curing and this confirms the chromosomal location of aerobactin determinants in the isolates of non compromised patients.

Hemolysins have tissue damaging properties which potentiate the bacterial toxicity and invasiveness[18], also increase the amount of iron available to the bacteria and liberate the iron that enhances the bacterial growth[19]. 35% of the tested isolates were hemolysin producers, also the isolates of non compromised patients express Hly as compared with those of compromised patients, and when the Hly is present chromosomal aerobactin is present, but when the Hly is absent plasmid aerobactin is present. Hybridazation studies have shown that several *E.coli* VF_s encoded by genes located on the same DNA gene cluster[20] and the deletion of one of them may also abolished the other[21] like aerobactin and Hly[1].

These findings support our observations, and the transposition events are responsible for such relationship[22]. We also conclude that the aerobactin plasmids of the isolates of compromised patients are relatively large ones and carry the determinants of either C or GM resistance. Bandereif and Neilands (1983)[7] estimated the size of aerobactin plasmid and called them P Col V plasmids in UPEC and found to be 144 Kb. In agreement with our observation, Perez- Casal and Crosa (1984)[5]reported that the determinants of some ABs are located on the aerobactin relatively large plasmids and associated with isolates of compromised patients. Johnson *et al* (1988)[1]explain such results by that the prior exposure of compromised patients to AB agents is responsible for increasing the resistance to some ABs in *E. coli* isolates from compromised patients. We believe that the presence of aerobactin operon composed of five genes[23]on the same plasmid with the determinants of resistance to one or more ABs make such plasmid a large one, and the presence of direct repeat at both ends of aerobactin genes is responsible for the transposition of aerobactin determinants to P Col V plasmids.

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