The Relationship between Phylogenic Typing and Antimicrobial Susceptibility Patterns for *Escherichia coli* Isolated from UTIs at Many Hospitals in Baghdad City

العلاقة بين الانماط الجينيه والمقاومه للمضادات الحيوية لبكتريا الايشيريشيا القولونيه المعزولة من المرضى المصابين بالتهاب المسالك البولية من عدة مستشفيات في مدينة بغداد

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المستخلص

الهدف: تهدف الدراسه الحاليه عزل بكتريا الايشيريشيا القولونيه من عدوى المسالك البولية في العديد من مستشفيات بغداد ودراسة مجموعة الانماط جينية وتقييم الارتباط بين المجموعات في عزلات بكتريا الايشيريشا كولاي مع نمط المقاومة للمضادات الحيوية التي تدرس باستخدام طريقة نشر القرص.

المنهجيه: تم جمع ٤٠٠ عزله من بكتريا الايشيريشيا القولونيه من نماذج البول للمرضى المصابين بالتهاب المجاري البوليه من خمس مستشفيات في مدينة بغداد وهي مستشفى غازي الحريري ،ابن البلدي،الاسكان ، النعمان ، و اليرموك. اجري التنميط الجيني لعزلات البكتريا .كافه باستخدام تقنية التفاعل التضاعفي لسلسله الدنا واستخدمت ثلاث بوادي لهذا الغرض. تحدد المجاميع الجينيه بالاعتماد على وجود ثلاث : قطع من الدنا أ chuA+, yjaA–, TspE4.C2+ ب١: chuA–, yjaA–, ب٢: chuA+, yjaA+, ومجموعة د chuA+, yjaA–. يجري فحص الحساسيه للعزلات البكتريه كافه ضد خمسة عشر مضاد حيوي من مجاميع مختلغه .

النتائج: تظهر الانماط الجينية لعزلات بكتريا ايشيريشيا القولون المعزولة من المرضى المصابين بالتهاب المسالك البولية كالاتي :النمط B2بنسبة ٤٣ ٪و ٢٨,٢٥٪ للنمط D و (٢٤٪) B1 وبالتالي كانت المجموعة B1 هي السائدة بين المرضى الذكور في مستشفى اليرموك وبنسبة (١٤٪)، تليها مستشفى الاسكان (٣,٧٥٪)، وكان معدل العزلة من *E.coli* أعلى في الفئة العمرية (١١-٢٠) سنة (٣١,٧٥٪)، وان نسبة (١١٪) منها كانت حساسية للمضادات الحيوية، في حين اظهرت (٧٥,٢٥٪) كممرضات مقاومة للأدوية واسعة النطاق، ولكن ١٣,٧٥٪ كانت مقاومة للأدوية المتعددة ، وفي مناطق الكرخ كان النمط الجيني B2 هو الاكثر ترددا وبنسبة (٥٩,٨٨٪)، يلية كلا النمطي الجيني A و) D ٦٨,٧٥٪ و (٤٨,٧٨٪) على التوالي مقارنة بمنطقة الرصافة حيث كان النمط B2 كان بنسبة (٤٠,١٢٪) ويلية كلا النمطين D و A و بنسبة (٣١,٢٥ و ٥١,٢٥)٪على التوالي ، وايضا فان النمط الجيني A كان اكثر ترددا لدى العزلات الاكثر حساسية المضادات الحيوية لكلا مستشفيات الاسكان و غازي الحريري وبنسبة ٪ (2.75, 2.5) على التوالي ، في حين نسبتة (٩، ٣,٧٥٪) على التوالي لدى العزلات المتعددة المقاومة للمضادات الحيوية في كل من مستشفى اليرموك و الاسكان .

التوصيات:تقترح الدراسة اجراء المزيد من الدراسات حول العلاقة بين الانماط الجينيه وانماط المقاومه للمضادات الحيويه لانواع اخرى من البكتريا او لنفس النوع من البكتريا معزوله من انواع اخرى من الاصابات ونوصي ايضا بتطبيق دراستنا هذه على بكتريا الكليبسيلا المعزوله من ادرار المرضى والذين يراجعون المستشفيات المشموله بالدراسه او في محافظات الوطن الاخرى .

Abstract:

Objective: The current study aime to isolate *Escherichia coli* from urinary tract infections (UTIs) in many Baghdad hospitals. The study concentrate on phylogenic groups and this was done based on triplex PCR method by primers besieged to three genetic markers, chuA, yjaA and TspE4.C2. Evaluate the relationship of phylogenic groups of *E. coli* isolates with the antibiotic-non sensitive patterns.

Methodology: Four hundred of *E.coli* bacteria isolated from urine samples from five hospitals in Baghdad city include: Ghazi AL-Hariri, Ibin- Al-Beledi , AL-Iskan , AL-Nooman and AL-Yarmoke hospitals. Phylogenetic categorization of

E. coli isolates was completed by by means of earlier reported triplex PCR-based on phylotyping procedure using primers besieged at three markers, *chuA*, *yjaA*and TspE4.C2. Phylogenetic combination was done on the starting point of the existence or nonexistence of the 3 DNA fragments as follows: *chuA*–, TspE4.C2–, group A; *chuA*–, *yjaA*–, TspE4.C2+, group B1; *chuA*+, *yjaA*+, group B2; *chuA*+, *yjaA*–, group D. Because two possible profiles can be obtained for the groups A, B2, and D. Fifteen antibiotics second-hand for all foremost groups and their individual generations were used in this paper adjacent to all bacterial isolates.

Results: The isolates of *E.coli* from UTI were distributed within the phylogroups B2 (43%); phylogroups D (28.25%); phylogroups A (24%) and B1 (3.5%), so phylogenic group B1 was prevalent among male patients of AL-Yermouk hospital (14%), followed AL-Iskan (3.75)% so, isolation rate of *E.coli* were higher among age (11-20) years (31.75 %), also(11%) were sensitivity to antibiotics, whilst (75.25%) were classified as extensive drug resistance pathogens, but (13.75%)were multidrug resistance and in Karkh areas, the frequency of phylogenetic B2 was (59.88%), followed phylogenetic A and D were (68.75 and 48.78%) respectively as compared to Rusafa areas were prevalence phylogenetic B_2(40.12%), followedphylogenetic D and A were (51.22 and 31.25 %) , as well as most of these phylogenetic groups occurred more frequently in both groups B2 or D (43, 28.25%) respectively, also (2.75, 2.5%) of phylogenic A were antibiotics sensitivity in both AL-Iskan hospital and Ghazi AL-Hariri hospital respectively whilst these phylogenic was multidrug resistance as percentage (9, 3.75) % respectively in both AL-Yermouk hospital and AL-Iskan hospital, Whilst in these hospital this phylogenic A was founded with extensive drug resistance (1, 0.75%) respectively, also most of phylogenetic group B2 occurred more frequently in AL-Yermouk hospital, so the sensitivity; multidrug resistance and extensive drug resistance as (2.25, 15 and 3.5%) respectively, also all phylogenetic groups *E. coli* isolates from UTI patients of five hospital weregrouped in two or three major group as A , B group contain major sub groups .

Recommendations: The study recommends additional studies of the correlation between the Phylogenic group with antibiotic-resistance pattern for other bacterial types isolated from UTIs or the same bacteria isolated from other types of infections. From this study, it is recommended to study on *Klebsilla spp* isolated from the infected patients urine who submitted to the same hospitals or in the other provinces of the country.

Key Words: Urinary tract infections, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), *E. coli*, Antibiotic resistance, MDR

Introduction:

One of the most common infections that affect humans is urinary tract infections (UTIs) [(1)]. More than 95% of UTIs cases causing by different microbes as bacteria; viruses and fungi. Bacteria are the majority agents Urinary tract infections (UTIs) [(2)] including equally gram -ve and gram +ve bacteria. The majority predominant common infecting of gram negative pathogens are *Escherichia coli* in percentage (50– 80%) of these infections.

The development of UTIs on numerous characteristics of organism and environmental factors, which needs of numerous virulence genes in the uropathogenic *Escherichia coli* (UPEC) which indomitable by the multitude genetic setting as well as useful and anatomic circumstances of the urinary tract, based on the strain's genetic contents *E. Couple* groups A and B1 are sister groups were as assembly B2 is incorporated in an inherited branch, these phylogroups it seems that contrast in their environmental niches and life-history.

In overall, generally UPEC strains fit in to the B2 cluster (and less frequently to group D), while the strains of the gut normal flora belong to groups A and B1 as non-pathogenic, even though the capability of *E. coli* to create UTIs are depended on numerous important different virulence factors like adhesions, capsule, toxins, and siderophores [(3).]

Antimicrobial agents are the most important chemical substances known and most powerful substances that produced by various microorganisms which used for killing or inhibit the susceptible bacteria that causing many infection in human [(4)],phenotypic testing of bacterial antimicrobial resistance or antibiogram is

one of the most commonly methods which between different isolates to many different antibiotics,but this type of test method is needed care for patients and it has widely used in diagnostic and clinical microbiology [5], so different isolates in susceptibilities can be considered different strains, as well as the first indication of outbreak of infections by identification of unusual or new pattern of antibiotic resistance among isolates cultured from multiple patients [6]. Multidrug resistance in *E.coli* strains can be developed a major concern, despite the occurrence tax for resistant strains of *E. coli* is considerably distinct from environments and a variety of populations consequently the collision of resistance to different antimicrobial drugs is everywhere [7].

Methodology:

Study population and bacterial isolation:

Examine populace and bacterial seclusion. In a cross-sectional study over a period of about 12 months, from April 2014 to April 2015, each of the 400 *E. coli* strains inaccessible from patients who experienced urinary tract disease in five hospitals in Baghdad city include: Ghazi AL-Hariri45(11.25%), Iben-Beledi 55(13.75%), AL-Iskan 73(18.25%), AL-Nooman 59(14.75%) and AL-Yarmok 168(41%) were subjected. The urine samples were cultured onto MacConkey agar (Oxoid) and blood agar (Oxoid) and incubated at 37°C overnight. Positive urine cultures were regarded as by the progress of a solitary settlement morphotype of *E. coli* with counts 105 CFU/ ml. *E. coli* was distinguished by criterion laboratory, methods, as well as morphological and biochemical reaction. These patients were of various age gatherings, sex groups and were inspected by standard techniques for bacteriology and biochemistry.

Genomic DNA extraction: Cultures grown for 18 hours in LB broth were utilized for DNA extraction was performed by utilizing used in bacterial typing, for comparison Wizard® Genomic DNA Purification Kit as indicated by the producer's proposals. After DNA extraction was finished, the DNA concentration of the samples was resolved with Quantus Florometer.

Application of PCR: With a specific end goal to affirm the isolates as *E. coli*, PCR test that in view of housekeeping gene rpsl) the sequence with particular primers as described by [8]. Were done in 25 L reaction volumes formed from 12.5 µl of GoTaq®Green Master Mix,template DNA 5µl, forward & reverse primers 1.5 µl for each, and 4.5 µl of Deionized Nuclease Free water was added to The PCR mixture to get the last volume of 25 µl. PCR mixture exclusive DNA template was utilized as a negative control. Under the following conditions the PCR was run: necessary denaturation step at 95°C for 5 min, 30 repetitive cycles start with the denaturation step at 94°C for 30 sec, annealing at 57°C for 30 sec, and 1 min at 72°C as extension step followed by the last extension step at 72°C for7min.

Phylogenetic categorization of *E. coli* isolates was completed by by means of a formerly research triplex PCR-based phylotypic technique [9]. Quickly, the amplification of specific regions in bacterial genomic DNA strains was done by triplex PCR technique using primers amplified three markers, *chuA*, *yjaA*and TspE4.C2. The primers used for PCR amplification were: ChuA (5`-GAC GAA CCA ACG GTC AGG AT-3`), YjaA (5`-TGAAGTGTCAGGAGACGCTG-3`), and TspE4.C2 (5`-GAG TAA TGT CGG GGC ATT CA-3`), which make 279-, 211-, and 152-bp amplicons, respectively[10]. The amplifications were accomplished in a total volume of 25 µl, every reaction mixture containing 2.5 µl 10X buffer (provided with *Taq*polymerase) (CinnaGen Co., Iran), 11.25 µl distilled water, 1 µldNTPs (each deoxynucleoside triphosphate at a 200 mm

concentration), 0.75 µl MgCl2, 2.5 U of *Taq* polymerase, 1 µl of each primer (20 pmol), and 3 µl of DNA template. Thermal cycler conditions were as following: initial denaturation at 95°C for 4 min for 30 cycles, denaturation at 95°C for 30sec, annealing at 48°C for 90sec, and a final elongation at 72°C for 1 min. Amplification yields were separated in 2% agarose gels containing ethidium bromide [10]. After electrophoresis, the gel was captured under UV light. The outcomes permitted the grouping of E. coli isolates to classify into one of the four main phylogroups (A, B1, B2, or D) [11]. Phylogenetic grouping was performed on the basis of the absence or presence of the three DNA fragments as follows: *chuA*–, TspE4.C2–, group A; *chuA*–, *yjaA*–, TspE4.C2+, group B1; *chuA*+, *yjaA*+, group B2; *chuA*+, *yjaA*–, group D. Because two possible profiles can be gained for the groups A, B2, and D, [12].

Drug sensitivity by disc diffusion method: A disc agar-diffusion technique, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (2013), was used to find out antibiotic sensitivity Fifteen antibiotics used for all major groups and their respective generations were used in this study. Drug sensitivity was determined using Kirby-Bauer method of disk diffusion for all isolates with CLSI guidelines [13] for discs: augmenten (AUG:20 µg), aztreonam (ATM:30µg), amikacin (AK: 30 µg), ciprofloxacin (CIP: 5 µg), Levofloxacin (LEV:5µg), trimethoprim/sulfamethoxazole (TS:1.25/23.75µg), Nitrofurantoin F:200µg), Ceftazidime (CAZ:30µg), imipenem (IPM:10µg) and Cefoxitin (FOX:30 µg), Cefotaxime (CTX:30 µg), Ceftriaxone (CRO:30 µg), Gentamicin (CN:10 µg), Nalidixic acid (NA:30 µg) and Norfloxacin (NOR:10 µg).

Results :

Table (1): Distribution of *E. coil* Phylogenic Typing According to the Hospitals.

Hospitals ware	Phylogenic classes (Number, %)				Untypable
	A	B1	B2	D	
Ghazi AL-Hariri	15(3.75)	1(0.25)	14(3.5)	13(3.25)	2(0.5)
Ibin- Al-Beledi	8(2)	3(0.75)	21(5.26)	22(5.5)	1(.25)
AL-Iskan	21(5.25)	3(0.75)	20(5.25)	29(7.25)	0(0)
AL-Nooman	7(1.75)	0(0)	34(8.5)	18(4.5)	0(0)
AL-Yermouk	45(11.25)	7(1.75)	83(20.75)	31(7.75)	2(0.5)
Total	96(24)	14(3.5)	172(43)	113(28.25)	5(1.25)

A total of 400 of *E.coli* bacteria were isolated from sample urine of patients suffering from Urinary tract infections (UTIs), According to phylogenic typing, *E.coli* isolated were confidential into four categories(A, B1, B2, and D) as well as untypable. These categories were outlined in the table 1 as the following: Greater percentage (43%) of isolates in the study belonged to phylogroup B2 and (28.25, 24%) for both phylogroup D and A respectively, but low percentage (3.5%) for B1,whilst study of [14] who reported that the predominant group of *E.coli* recovered from UTI patients in their study belonged to phylogroup D, so in this study show (1.25%) was non-typable (table 1 and figure 1).

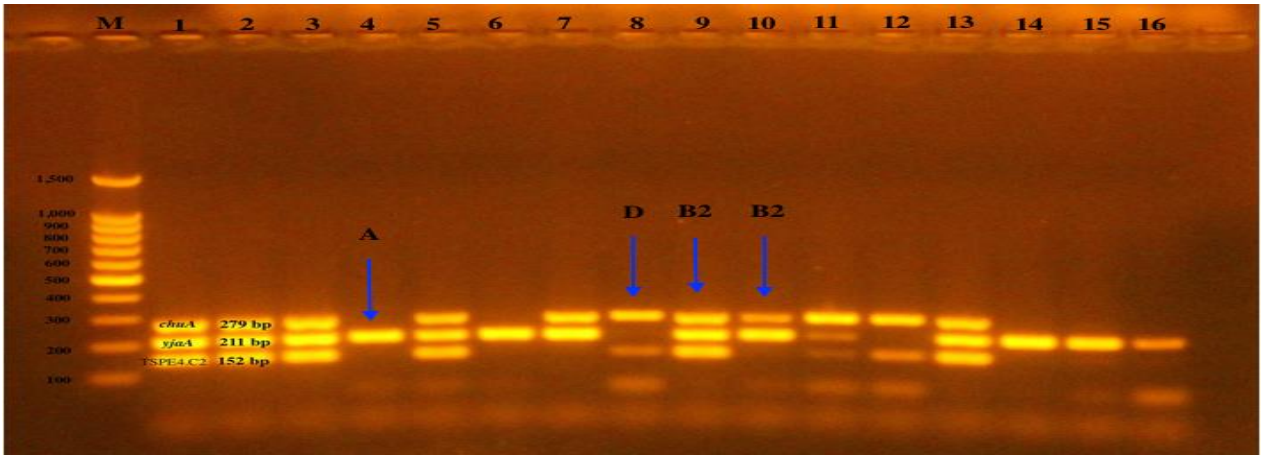

Figure (1): Triplex PCR based phylogenetic sketch of *E.coli* isolates. path M contained 100 bp marker; Lane 1(EC1), Lane2 (EC2), Lane3 (EC3), Lane4 (EC4), Lane5 (EC5), Lane6 (EC6), Lane7 (EC7), Lane8 (EC8), Lane9 (EC9), Lane10 (EC10), Lane11(EC11), Lane12 (EC12), Lane13 (EC13), Lane14 (EC15), Lane16 (EC16)

Phylogenetic grouping was determined in this study for all isolates by using triplex PCR reaction targeting three DNA markers (chuA, yjaA and TSPE4.C2). The different banding patterns of *E. coli* strains help in classifying or segregating them into one of four major phylogenetic groups (A, B1, B2, and D) regarding the presence and absence of genes (*chuA*, *yjaA*, and TspE4). That means the *chuA* gene was existent in the strains of groups B2 and D and was none existent from the strains of groups A and B1. This made it easier to separate groups B2 and D from groups A and B1. Besides, the *yjaA* gene permitted

Table (2): Distribution of Phylogenic Typing of *E.coli* According to Age and Gender

Phylogenetic type	Sex		Age groups (years)							Total
	Male	Female	≤ 10	11-20	21-30	31-40	41-50	51-60	>60 years	
A (%)	76 (19)	20 (5)	19 (4.75)	5 (1.25)	29 (7.25)	18 (4. 5)	11 (2.75)	10 (2. 5)	4 (1)	96 (24)
B1 (%)	12 (3)	2 (0.5)	3 (0.75)	2 (0.5)	4 (1)	1 (0.25)	4 (1)	0 (0.25)	0 (0.25)	14 (3.55)
B2 (%)	132 (33)	40 (10)	22 (5.5)	16 (4)	50 (12.5)	39 (9.75)	24 (6)	17 (4.25)	4 (1)	172 (43)
D (%)	57 (14.8)	2 (0.5)	26 (6.5)	2 (0.5)	43 (10.75)	16 (4)	20 (5)	4 (1)	2 (0.5)	113 (28.25)
Non-typable (%)	4 (1)	1 (0.25)	0 (0)	0 (0)	1 (0.25)	2 (0.5)	0 (0)	2 (0.5)	0 (0)	5 (1.25)
Total (%)	325 (81.25)	75 (18. 75)	0 (0)	70 (19.25)	25 (6.25)	127 (31.75)	76 (19)	59 (14.75)	33 (8.25)	10 (2. 5)

Results in table (2) showed the phylogenic group B1 was prevalent among male patients are a from AL-Yermouk hospital (14%), followed AL-Iskan (3.75%) whilst Lower proportion of males were in Ghazi AL-Hariri Hospital (3%) , as well as the males were more than females in all other phylogenic (A,B1,B2,D) in the five hospitals (Ghazi AL-Hariri ; Ibin Al-Beledi ; AL-Iskan ; AL-Nooman and AL-Yermouk) as percentage (19, 3,33,14.8%) respectively when compare the female in the same phylogenic and hospital (5, 0.5 , 10, 0.5%) respectively.

In this study the other important factor is age, the isolation rate of *E.coli* were higher among age (11-20) years (31.75 %), and nearly same percentage (19.25 &19%) of the isolates were from both age groups (≤10 and 21-32) years, so the high prevalence of most phylogenic type (A, B2 and D) as (7.25, 12.5 and 10.75%) respectively in age group (21-30) years, whilst increased prevalence the phylogenic B1 in age group (41-50) years**.**

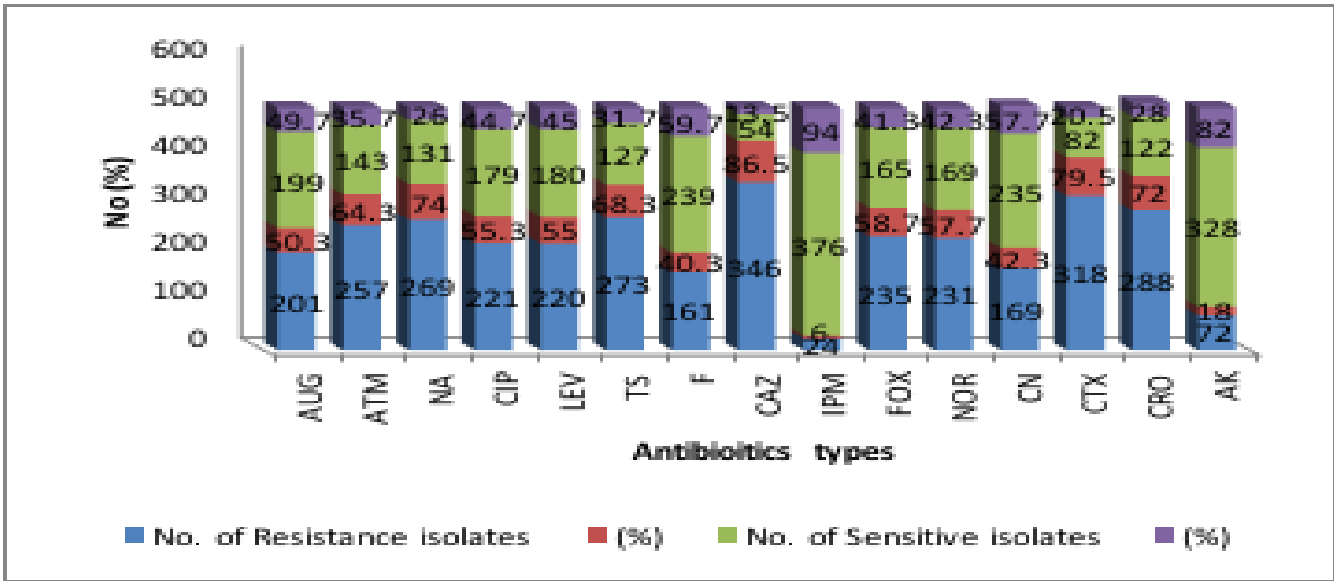

Figure (2): Antibiotic Sensitivity Patterns of *E. coli*

Figure (2) shows that *E. coli* exhibited in this study a resistant rate of > 70% to nalidixic acid, cefotaxime, ceftazidime, and ceftriaxone,. However, E. coli demonstrate a low resistance to imipenem (6%), nitrofurantoin (40.25%), gentamycin (42.25%), and amikacin (79.4%). Likewise, a modest resistant range (50 to 70%) was shown by E. coli to augmentin, aztreonam, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxzole, and cefoxitin. The current search shows that *E. coli* isolates were extremely susceptible to imipenem, amikacin and nitrofurantoin, a detection that was consistent to that reported by others (4,5). In addition, this research showed resistance rate of 55.25%, 57.75% and 79.4% to ciprofloxacin, norfloxacine and gentamycin correspondingly, which is lesser to account by others (6,7). Besides, high confrontation rate of urinary *E. coli* isolates to levofloxacin (67.4%) which was superior to that reported before. (8,7). A nalidixic confrontation of *E. coli* isolates was elevated (74%). In addition, E. coli resistance rate trimethoprim was 68.25% and this with health brunt since this drug is the first line up FOR treatment record of UTI. Aztreonam resistance of *E. coli* isolates was 64.25%.

Table (3): Distribution of *E.coli* Phylogenic Typing with Resistance Pattern in All Hospitals

phylogenic type	Sensitive (No.%)					MDR(No.%)					XDR(No.%)					Total (No. %)
	G.H	I.B	A.N	A.I	A.Y	G.H	I.B	A.N	A.I	A.Y	G.H	I.B	A.N	A.I	A.Y	
A	4 (1)	0 (0)	2 (0.5)	3 (0.75)	5 (1.25)	11 (2.75)	8 (2)	5 (1.25)	15(3.75)	36 (9)	0 (0)	0 (0)	0 (0)	3 (0.75)	4(1)	96 (24)
B_1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.25)	2 (0.5)	0(0)	2 (0.5)	6 (1.5)	0 (0)	1 (0.5)	0 (0)	1 (0.25)	1 (0.25)	14 (3.5)
B_2	3 (.75)	3 (.75)	5 (1.25)	1 (0.25)	9 (2.25)	11 (2.75)	15 (3.75)	27 (6.75)	14(3.5)	60 (15)	0 (0)	3 (0.75)	2 (0.5)	5 (1.25)	14 (3.5)	172 (43)
D	0 (0)	4 (1)	2 (0.5)	1 (0.25)	5 (1.25)	9 (2.25)	19 (4.75)	15(3.75)	25(6.25)	16 (4)	4 (1)	2 (0.5)	1 (0.25)	3 (0.75)	10 (2.5)	113 (28.25)
Untapable	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	2 (0.5)	1 (0.25)	0 (0)	0 (0)	1 (0.25)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.25)	5 (1.25)
Total	7 (1.75)	9 (2.25)	9 (2.25)	5 (1.25)	19 (4.75)	34 (8.5)	45 (11.3)	47 (11.8)	56(14)	119 (29.75)	4 (1)	6 (1.5)	3 (0.75)	12 (3)	30 (7.5)	400 (100)
Sum (%)	44(11%)					301(75.25%)					55(13.75%)					

NOTE: G.H: Ghazi AL-Hariri hospital I.B: Ibin Al-Beledi hospital , A.I: AL-Iskan hospital, A.N: AL-Nooman hospital.; A.Y: AL-Yermouk hospital, S: sensitivity ; MDR: multidrug resistance , XDR: Extensive drug resistance

Multidrug resistance which was characterized as resistance to 3 or more types of the antibiotics (Canton R and Ruiz-GarbajosaP (2011). A total of 44 isolates (11%) were sensitivity to antibiotics that using in this study, whilst 301 isolates (75.25%) were classified as extensive drug resistant pathogens, but 13.75% were multidrug resistance were presented in tables 3.

The spreading of phylogenetic collection (A, B1, B2 and D) among fivehospital (Ghazi AL-Hariri; Ibin Al-Beledi; AL-Iskan hospital; AL-Nooman hospital and AL-Yermouk) in all strains of *E.coli* were examined (Table 3). Most of these phylogenetic groups occurred more frequently in both groups B2 or D (43, 28.25) % respectively, also this table showed (2.75, 2.5)% of phylogenic A were antibiotics sensitivity in both AL-Iskan hospital and Ghazi AL-Hariri hospital respectively whilst these phylogenic was multidrug resistance as percentage (9, 3.75) % respectively in both AL-Yermouk hospital and AL-Iskan hospital, Whilst in these hospital this phylogenic A was founded with Extensive Drug Resistance (1, 0.75) % respectively.

In addition most of phylogenetic group B2 occurred more frequently in AL-Yermouk hospital, so the sensitivity; multidrug resistance and extensive drug resistance aspercentage (2.25, 15 and 3.5) % respectively, whilst most more frequently in AL-Nooman hospital to sensitivity, multidrug resistance as percentage (1.25, 6.75 and 3.5) % respectively,but 1.25 as extensive drug resistance in the same hospital, also phylogenetic group B1 occurred more frequently in both AL-Iskan hospital and Ibin Al-Beledi hospitalas multidrug resistance and extensive drug resistancein percentage (0.5%), whilst phylogenetic group B2 washigh percentage (1, 0.75) % respectively asmultidrug resistance and extensive drug resistance, whilst most belonging to phylogroup B1 occurred more frequently as MDR and XDR (0.5%) in both AL-Iskan hospital and Ibin Al-Beledi hospital respectively, as well as phylogenetic group D was high percentage (1.25%) as Sensitivity,while (4.75,6.25)% respectively as multidrug resistance in both Ibin Al-Beledi hospital and AL-Iskan hospital, but low percentage as (2.5 and 1)% as extensive drug resistancein both Ghazi AL-Hariri hospital and AL-Yermouk hospital.

Table (4): Relationship Between Antimicrobial Susceptibility Patterns and Phylogeny Type in *E.coli* Strains

Phylogenic type	Sensitive (%)	*MDR (%)	XDR (%)	Total %
A	14(3.5)	75(18.75)	7(1.75)	96(24)
B1	0(0)	11(1.75)	3(1.75)	14(3. 5)
B2	21(5.25)	127(31.75)	24(6)	172(43)
D	9(2.25)	84(21)	20(5)	13(28.25)
Untypable	0(0)	4(1)	1(0.25)	5(1.25)
Total (%)	44(12%)	301 (75.25%)	55 (13.75 %)	400(100)%

*MDR: : multidrug resistance , XDR: Extensive drug resistance

The results found high percentage (75.25%) of isolations are multiple drug resistance whilst (13.75%) of the isolates are extensive drug resistance and only (12%) of the isolates are sensitive to fifteen antibiotics.

Table (5): Comparative between Karkh and Rursafa According to Phylogenetic Type

Phylogenetic type	Karkh	Rusafa	Total	P- value
A	66(68.75)	30(31.25)	96	0.001
B1	10 (71.42)	4(28.57)	14	0.001
B2	103(59.88)	69(40.12)	172	0.001
D	60(48.78)	63(51.22)	123	0.06 (NS)

In Karkh areas, the frequency of Phylogenetic B2 was (59.88%), followed phylogenetic A and D were (68.75 and 48.78%) respectively as compared to Rusafa areas where prevalence phylogeneticB2 (40.12%), followed phylogenetic D and A were (51.22 and 31.25 %) respectively (Table 5). In addition, the overall frequency of Phylogenetic B1 in Karkh was found to be 71.42% as compared to Rusafa areas where prevalence Phylogenetic D as (51.22%), also in totally high prevalence of phylogenetic B2 (No.= 172) ,followed Phylogenetic D (No.= 123).

Discussion

E.coli isolates were confidential into four categories(A, B1, B2 and, D) as well as non-typable, greater percentage (43%) of isolates in the study belonged to phylogroup B2 and (28.25, 24) % for both phylogroup D and A respectively.

In a classification correlational study , most common of *E.coli* isolate from UTIs are phylogroup B2 (38.3%) following by phylogroup A; D as (28.3, 26.3)%, and (7.2%) for group B1[18] . The phylogenic group B1 was more prevalent among male patients of AL-Yermouk hospital followed AL-Iskan whilst Lower proportion of males were in Ghazi AL-Hariri Hospital, in addition to the males were more than females in all other phylogenic (A,B1,B2,D) in the five hospital (Ghazi AL-Hariri, Iben- Beledi, AL-Iskan, AL-Nooman and AL-Yermouk). Concerning to this finding, new article recognize that gender are ominously connects with the phylogenetic A, however, only females who pass out the *E. coli* isolates fit in to these phylogenetic A, as well as elderly are more than young groups who carriers *E.coli* isolates belonging to the phylogenetic D, [19] while no differences for both phylogenetic (B1and B2) in different age and gender groups[20].

Another study has found that the elderly males are a greater isolated of *E. coli* isolates than both young male and females, [20] explained by the changes in surfaces in the elderly. In addition correlated study showed that all age groups, except young males, that have *E. coli* belonging to phylogenetic D, as the majority of population, wherase second dominant group of phylogenetic for the elders are B2, while phylogenetic group D which carrie by *E.coli* strains for young females related to group A. (75.25%) were as multiple drug resistance pathogens, whilst only 13.75% as extensive drug resistance and only (12 %) of the isolated strains were sensitive to antimicrobials test [21]. The results agree with these findings of Sabir *et al*., who shows that MDR and XDR are 81% and 8.7% respectively to *E. coli* which isolate from UTIs [15]. The findings of this study are pertain with the findings of others researchers [16,17]. Collateral search demonstrates that the isolates of *E. coli* have widespectrum antibiotic resistance, so contaminated with antimicrobial resistant *E. coli*, like of animal origin *E.coli* can be colonized in humans because of it resistance to normally extensive range of antimicrobial agents, and these bacteria can cause infection with limited available therapeutic [21,22,23].

A greater percentage of isolates in the study belonged to phylogroup B2 and both phylogroup D and A respectively.phylogenic group B1 was prevalent among Male patients from AL-Yermouk hospital, followed AL-Iskan whilst Lower proportion of males were in Ghazi AL-Hariri Hospital, as well as the males were more than females in all other phylogenic (A, B1, B2, D) in the five hospitals (Ghazi AL-Hariri, Iben- Beledi, AL-Iskan, AL-Nooman and AL-Yermouk.Yermouk). Isolation rate of *E.coli* were higher among age (11-20) years , and nearly same percentage of the isolates were from both age groups (≤10 and 21-32) years, so high prevalence of most phylogenic type (A, B2 and D) respectively in age group (21-30) years, whilst increased prevalence the phylogenic B1 in age group (41-50)years**.**High percentage of isolated were multiple drug resistance whilst low Percentage of the isolates were extensive drug resistance. In Karkh areas, high

frequency of Phylogenetic B2, followed phylogenetic A and D respectively as compared to Rusafa areas were prevalence phylogenetic B2, followed phylogenetic D and A.phylogenic A were antibiotics sensitivity in both AL-Iskan hospital and Ghazi AL-Hariri hospital respectively whilst these phylogenic was multidrug resistance in both AL-Yermouk hospital and AL-Iskan hospital, While in these hospital this phylogenic A was founded with Extensive Drug Resistance as well as most of phylogenetic group B2 occurred more frequently in AL-Yermouk hospital. High rates of MDR and EDR to antimicrobial to *E. coli* isolate.

Recommendations:

The study recommends additional studies of the correlation between the phylogenic group with antibiotic-resistance pattern for other bacterial types isolated from UTIs or the same bacteria isolated from other types of infections. We suggest to applicate our study on bacteria *Klebsilla spp* isolated from the infected patients urine who submitted to the same hospitalsor in the other provinces of the country.

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