Phenotype and Genotype of Virulence Factors in Escherichia Coli Isolated from Different Clinical Samples

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Abstract

Escherichia coli is the principal causative agent of most infection of bacterial origin, and extraintestinal pathogenic E. coli (ExPEC) is responsible for 80% of infections of urinary tract in human bieng. The aim of this work was to study the distribution of some virulence factors of Escherichia coli in different type of human samples. This study was carried out in Baghdad city-Iraq from January to June 2019, included 70 clinical samples (Blood, Urine and Wound samples) with positive culture for E. coli. Each sample was managed bacteriologically to isolate causative bacteria as mentioned in standard systems of bacteriology Lab. The DNA was extracted from all E. coli isolates for detection of fim H and Kpc gens (important virulence factors of bacteria) by PCR and for determination resistance antibiotics these isolates. The present study showed that the highest rate of E coli isolates (78.57%) was from urine samples of patients with UTI and the lowest percentage was from blood samples (8.57%). The study show highest percentage of E coli isolates (95.43%) was positive for adhesion factors, followed by 91.43% was positive for biofilm formation, 70% was capsule producer, while 21.43% of E coli isolates was multidrug resistance (MDL) (p: < 0.05). The study revealed that 95.71% of E coli isolates was resistant for cefotaxime, 94.29% was resistant to aztroneom while 2.86% was resistant to amikacin. The study revealed that 95.71% of E coli isolates was with adhesion factors and 70% of E coli isolates capsule producer as phenotypic virulence factor while 95.71% of E coli isolates was positive for Fim H gene and 4.29% was positive for Kpc gene (as genotype virulence factors).

Conclusions: nearly quarter of E coli isolated was multidrug resistant and fim H virulence gene was the most gene detected in those isolates

*Keywords***:** virulence factor; E coli; Fim H gene, Kpcgene MDL.

النمط الظاهري والتركيب الوراثي لبكتريا E.coli القولونية المعزولة من عينات سريرية مختلفة النمط

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الخلاصة

الإيشـريشـا القولونيه هي احد العوامل المرضـية و كمسـبب رئيسـي لمعظم حالات العدوى البكتيرية ، حيث تعتبر هذه البكتريا نوع (extraintestinal pathogenic E. coli (ExPEC) المسؤولة عن 80 ٪ من التهابات المسالك البولية , لذا هدفت الدراسة الحالية لدراسة المظهرية و الجنينية لعوامل ضراوة البكتريا المعزوله من عينات سريرية مختلفة. وقد أجريت هذه الدراسـة في مدينة بغداد - العراق في الفترة من يناير إلى يونيو 2019 ، وشـملت 70 عينة سـريرية (عينات دم وبول ومسـحات الجروح) وقد زرعت البكتريا وشـخصـت بطرق كلاسـيكية وحديثة وقد اسـتخلص الحامض النووي, و كشـف عن جينات الضـراوه fim H و Kpc باسـتخدام . (PCR) أظهرت الدراسـة الحالية أن أعلى نسـبة لعزلات البكتريا (78.57 ٪) كانت من عينات البول لمرضـى التهاب المسـالك البولية وأدنى نسـبة كانت من عينات الدم (8.57 ٪). أظهرت الدراسـة أن أعلى نسبة من عزلات E coli (95.43٪ (كانت إيجابية بالنسبة لعوامل الالتصاق ، تليها 91.43٪ كانت إيجابية لتكوين الغشـاء الحيوي ، 70٪ كانت منتجة للكبسـولة ، بينما كان 21.43٪ من عزلات E coli مقاومة متعددة الأدوية .(0.05> : (p (MDL) كشـفت الدراسـة أن 95.71٪ من عزلات E coli كانت مقاومة للسيفوتاكسيم ، 94.29٪ كانت مقاومة للأزترونوم بينما 2.86٪ كانت مقاومة للأميكاسين. و أن 95.71٪ من عزلات E coli لها قابلية التصـاق وأن 70٪ منها كانت منتجة للكبسـولة كعامل ضـراوة النمط الظاهري بينما كان 95.71٪ منها كانت إيجابية لجين Fim H و 4.29٪ كانت إيجابية لجين Kpc وقد اسـتنتج ان ما يقارب ربع العزلات كانت متعددة المقاومَة للأدوية المتعددة وكان الـ H fim هو أكثر الجينات المكتشفة في تلك العزلات.

الكلمات المفتاحية : عوامل الضراوه , بكتريا الاشيرسيا القولونية , جينات الضراوه , (Kpc , H fim) متعددة المقاومَة للأدوية.

Introduction

E. coli is the principal agent causative of most infection of bacterial origin, and extraintestinal pathogenic E. coli (ExPEC) is responsible about 80% of infection of urinary tract [1]. Furthermore, 90% of UTIs causing by theUropathogenic type of E. coli (UPEC) which occurred in all ages [2]. Bacteria from abdomen (fecal origin) with ascending infections, live in the urethra and spread up to the bladder and to the kidneys resulting in pyelonephritis or prostitisis in men [3]. Because the shorter urethra of women, they predominantly suffered from UTI 14 times more than males [1]. Moreover, the high rate of extended-spectrum β-lactamases (ESBLs) occurrence in ExPEC infected patients, like those infected with E. coli have the C.T.X-M enzymes, which repsonsable of true problems in treatment [4]. Likewise, from the time when the first study of CTX-M-harbor Enterobacteriaceae. This situation could have moved clinicians and researcher to use of up-to-date antibiotics, like AB drugs to treat numerous severe infections.

Therefore, this could have designated bacteria with novel genes which may act in degradation of carbapenem group of antibiotics, this is finest example in using NDM-1 gene [5,6].

Subsequent the first report of NDM-1 gene in Klebsiella pneumoniae in last decade, a diversity of bacterial species reported globally give positive result for NDM-1 carbapenemases [1]. The development and continuous appearance of antimicrobial-resistant is thus the universal problem. The increase in the occurrence of multidrug-resistant (MDR) bacteria in last year's reflex a major public health problem [7]. Furthermore, the spreading of clonal organisms carrying a newly antibiotic resistance gene has worse the problem [2]. The strains with 131 sequence type form a wide spread clone which is quickly and continuously disseminating in diverse countries globally. Clinical new studies also proposed that the strains belonging to this set are extremely virulent with capability benefit [8]. UPECis the first public bacterial pathogens caused (70-90%) of community-acquired UTI and (40-50%) of nosocomial UTI in a different age groups [9]. The distinct form of UPEC strains, are from the commensal E. coli exist in in the abdomen encode a many of virulence factors, which have a significant role in bacterial infection and colonization in the urinary tract [4]. Adherence factors like (afimbrial adhesins ;P fimbriae; S and F1C fimbriae;and type 1 fimbriae) is the most important VFs related with UPEC strains which including (α-hemolysin and cytotoxic necrotizing factor type 1) [10]. The aim of this work was to study the distribution of some virulence factors of Escherichia coli in different type of human samples.

Materials and methods

These study was carried out in Baghdad City-Iraq from   January to June 2019, included 70 samples with positive culture for E. coli, samples distribute as follows:

Type of samples	No.
Blood	6
Urine	55
Wounds & burns	9

Each sample was managed bacteriologically to isolate causative bacteria as mentioned in standard systems of bacteriology Lab, the isolated bacteria the fully isolated by sub culture on MacConkey agar and biochemical tests were done. Kirby-Baur methods was applied for all isolates and bacteriological procedure were also done of pure colonies of isolates for identification of their virulence factors.

Capsule production: It is a way of using wet amount by putting one drop from Indian ink on surface of a clean slide and using the loop to transport a part of culture (grown on brain heart infusion broth). The part of culture was mixed with Indian ink on slide and covered by cover slip between two filter papers (to prevent aggregation of colony). The slide was examined under the light microscope. The formation of a clear halo unstained with Indian ink around microorganism indicated the presence of capsule [10].

DNA extraction

The DNA was extracted from all E. coli isolates. E. coli DNA was with good quantitative and qualitative states that showed one band of DNA when analyzed by the gel electrophoresis method, the purity of extracted DNA was between 1.7 and 1.9.

PCR study

The knowledge of virulence features of different microorganism causing human infection will aid the clinician to expect and decide which bacteria infected their patients. The (eppendorf thermocycler Co) Polymerase Chain Reaction (P.C.R) was using in this study for detecting the occurrence of virulence genes Kpc and fimH using Specific primers to amplify the genes encoding virulence factors.

Statistical analysis

Computerized program for analysis was made using Mintab ver 19.0 statistic program for concluding of P. value (P<0.05: significant).

Findings

The study presented that the maximum rate of E coli isolates (78.57%) was from urine specimen UTIs patients and the lowest rate was from blood cultures (8.57%),(Table 1).

Table 1: Distribution of E coli isolates from different clinical samples

Type of samples	No.	%
Blood	6	8.57
Urine	55	78.57
Wounds & burns	9	12.86
Total	70	100

The study showed that the highest percentage of E coli isolates (95.43%) was positive for adhesion factors, followed by 91.43% was positive for biofilm formation, 70% was capsule producer, while 21.43% of E coli isolates was multidrug resistance (p: < 0.05) (Table 2).

Table 2: Distribution of E coli isolates according to their virulence factors

Virulence factor	Positive		Negative		P. value
	No.	%	No.	%	
Capsule production	49	70	21	30	<0.05
Adhesion factors	67	95.71	3	4.29	<0.05
Biofilm formation	64	91.43	6	8.57	<0.05
Multidrug resistance	15	21.43	55	78.57	<0.05

The study revealed that 95.71% of E coli isolates was resistant for cefotaxime, 94.29% was resistant to aztroneom while 2.86% was resistant to amikacin, Table 3.

Table 3: Distribution of antibiotic resistance E coli isolates.

Antibiotics	Sensitive		Resistance	
	No.	%	No.	%
Ciprofloxacin	54	77.14	16	22.86
Ceftriaxone	7	10	63	90
Aztroneom	4	5.71	66	94.29
Imipenem	63	90	7	10
Nitrofurantoin	61	87.14	9	12.86
Cefotaxime	3	4.29	67	95.71
Pipracillin	10	14.29	60	85.71
Amikacin	68	97.14	2	2.86
P. value <0.05				
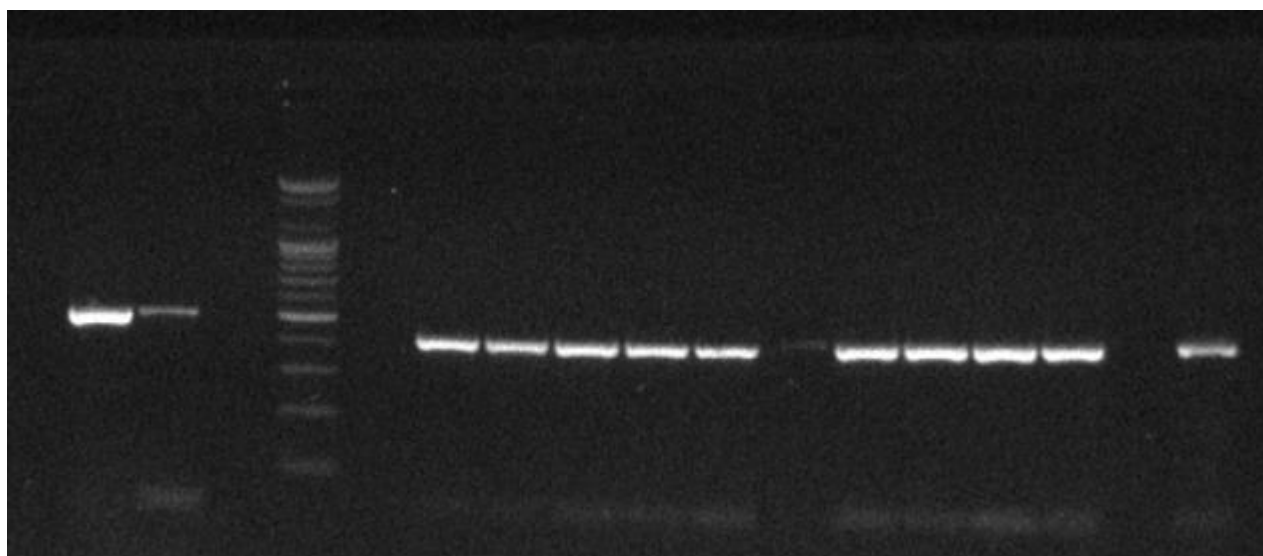
Figure (1): Ethidium bromide stained agarose gel showing PCR amplification products with FIM gene (508 bp) primers and Kpc gene (7988 bp) primers for E coli

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genotype and phenotype factors of virulence.

The study revealed that 95.71% of E coli isolates was with adhesion factors and 70% of E coli isolates capsule producer as phenotypic virulence factor while 95.71% of E coli isolates was positive for FIM gene and 4.29% was positive for Kpcgene (as genotype virulence factors), Table 4 and fig (1).

Table 4: Distribution of antibiotic resistance E coli isolates according to genotype and phenotype factors of virulence.

Virulence factor		Positive		Negative		P. value
		No.	%	No.	%	
phenotype	**Capsule production**	49	70	21	30	<0.05
	Adhesion factors	67	95.71	3	4.29	<0.05
Genotype	*Kpc*	63	4.29	7	95.71	<0.05
	Fim H.	67	95.71	3	4.29	<0.05

Discussion

Several studies were done clarified the prevalence of E. coli in clinical sample, Hamdoon [11] and Akter et al [12] found that urine of patients with UTI was the most source of E. coli in Medical laboratories. The main cause for 85% community-acquired urinary tract infection (UTIs) and 50% nosocomai UTIs is E. coli bacteria [1,2,5]. There are different factors which affect the prevalence of UTIs like gender, age, urological instruments, and immunosuppression (3,5). There are earlier studies Amin et al., [28] isolated E. coli from UTI showed the extreme degree of resistance to ampicillin and tetracycline while showed sensitivity to gentamicin, ceftriaxone, amikacin and ciprofloxacin. Bashir et al [13] reported that E. coli resistance to ampicillin, Ciprofloxacin, Nitrofurantoin, Co-Trimoxazole, Amikacin. Niranjan et al [14] express that E. coli in India, were more resistant to ampicillin, augmentin, ceftriaxone, cefuroxime and methoprim and were susceptible to amikacin, tazobactam&piperacillin; nitrofurantoin and imipenem. Although, from the present study the following antibiotic Peracillin+Tazobactam, Cefoperazone+sulbactam, Amikacin, Fosfomycin, Nitrofurantoin, Imipenem were used. In both research study, the same antibiotic was used and found good results. The occurrence of multi drug resistance (MDR) represent some foremost problems in the managing of pathogenic bacteria in UTI patients. Moreover, it has been observed that E. coli in clinical isolates of ESBL makers are further possible to be resistant to other non β-lactam antibiotics. This may be via plasmid carried numerous genes coding multiresistance that are transported from one bacteria to others. The forthcoming management of MDR ESBL generating

E. coli may developed more complex since of additional restrictions of the offered drugs. In our resulst, MDR made 21.43% of the isolates, where all are β-lactamase creators. Aminzadeh, et al [15] described 25% of E. coli was MDR. Other revisions stated much higher rates from 69.6% to 90.5% of E. coli with MDR ESBL-genes [3,16]. In fact, detection of the resistance genes of causative bacteria can aid in unlimited deal to choice the top antibiotic in the best time. Abdul-Ghaffar et al [17] Founded that 95.23% of E. coli isolated from UTI patients was with fimH gene. Wang et al [18] in his study on role of virulence factors of E. coli and host in the progress of E. coli bacteremia in patients with UTI. Abdul-Ghaffar et al [17] demonstrated that there was a high rate of the genetic factor (92%) of type 1 fimbrial adhesin (fimH). While in a total of 78 E. coli strains isolated from diverse kinds of urinary tract infections in Romani were screened for fimH adhesion gene and they found with rate 86% [19]. A similar result showed rate 86.1% of fim H virulence gene as recorded by Manjarrez-Hernandez et al [20] revealed that in 108 E coli isolates from Mexican women with urinary tract infection, were studied for the presence and identification of virulence genes, phylogenetic groups, and antibiotic resistance. Thus, uropathogenic strains of E. coli are believed to display a variety of virulence determinants Goh et al [20] revealed that the most virulence factors detected in E coli were: siderophores, toxins, capsules, fimbriae and others have been described. The principal role of the polysaccharide capsule is consequently studied in different reports dealt with UPEC virulence [21,22]. The prototypical group capsule offers defense wall against phagocytosis and complement line, gives a role in immune evasion through molecular mimicry, and further surface-associated antigens [21,23].

Conclusions

nearly quarter of E coli isolated was multidrug resistant and fim H virulence gene was the most gene detected in those isolates

Conflict of interest: non

Source of findings: Hospital

Ethical clearance: This research was carried out with the patient's verbal and analytical approval before the sample was taken.

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