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Involvement of Virulence Genes and Antibiotic Resistance in Clinical and Food Borne Diarrheagenic *Escherichia coli* Isolates From Egypt

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Abstract: A total of 140 *E. coli* isolates showed at least one pathogenic determent among the isolated 147 *E. coli* which were previously collected from clinical and food samples. The other 7 isolates were free from tested virulence genes. Among these 140 isolates, 123 had only the *UidA* gene, therefore, were classified as non-categorized pathogenic *E. coli*. The other 17 isolates had at least one characteristic gene of diarrheagenic *E. coli* and were identified and classified as enteropathogenic *E. coli* (8), enterotoxigenic *E. coli* (5) and enterohemrrahagic *E.coli* (4). Interestingly, a correlation was found among these isolates that increasing the number of virulence genes was accompanied with an increase in the antibiotic resistance until a cutoff of 2 virulence gens, above this limit the relation was inversely proportion. Evidentially, sensitive K12 *E. coli* was able to acquire ampicillin and/or kanamycin resistance traits from resistant pathogenic *E. coli* isolate among those collected in the present study. NSEM monitoring of different growth stages of the mated K12 *E. coli* and the donor resistant pathogenic *E. coli* showed that horizontal gene transfer by conjugation occur during the growth, suggesting that this was one of possible mechanisms through which the antibiotic resistance characters were transferred or acquired.

Key words: Acquisition · Conjugation · *E. coli* · Multiplex PCR · Resistance

INTRODUCTION

Historically, the effects of *E. coli* on morbidity, mortality and healthcare were not a major source of concern, since these microorganisms were eradicated with simple antibiotic therapy. Unfortunately, the situation has changed dramatically in the last two decades. Recently, the highest attention was grasped to *E. coli* after reporting the newly emerging pathotypes such as entero aggregative EHEC O104:H4 strain as the causative agent in a large outbreak of hemolytic colitis and hemolytic-uremic syndrome in Germany and other European countries [1, 2].

No doubt that the emergence of new pathotypes of *E. coli* with new virulence factors coded for serious diseases is not the only problem, as the problem of antimicrobial resistance has also been increased in the last decade and is a major public health threat worldwide [3, 4]. Matter of fact, antimicrobial resistance has been found in pathogenic and non-pathogenic strains as it act as a reservoir of antibiotic resistance genes for human pathogens [5].

Therefore, more studies have been focused on the emergence of severely virulent and/or resistant *E. coli* members [6]. Indeed, *E. coli* members are well known as the most versatile bacterial species and the diversity of its lifestyles is achieved through a high degree of genomic plasticity, via gene loss or gain, through horizontal gene transfer (HGT) [7]. The antibiotic resistance and virulence determinants share some basic characteristics, where both have been reported to be acquired by means of HGT from organism to another [8]. Hence, HGT plays a key role in the evolution of bacteria and the spread of antimicrobial resistance genes [9]. It involves the acquisition by the bacterial cell of a foreign DNA, a phenomenon that may occur via three common mechanisms; transformation (capture of free DNA), transduction (via bacteriophage DNA), or conjugation [10].

In this prospective, this study was conducted to identify relevant pathogenic *E. coli* from both clinical and food samples. These isolates were screened for commonly known virulence genes of diarrheagenic *E. coli* (DEC) through 5 schemes of PCR reactions. The antimicrobial resistance of these isolates was previously determined [4].

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The correlation between antimicrobial resistance and virulence genes was investigated. Moreover, the ability of acquiring antibiotic resistance traits from resistant *E. coli* to susceptible *E. coli* isolates was documented. Eventually, more work has been pursued to elucidate the main possible mechanism of such acquisition of antibiotic resistance characteristics.

MATERIALS AND METHODS

Unless otherwise, specified all tests were conducted under aseptic conditions and in triplicates. The represented data equal mean ± standard deviation of conducted replicates.

Microorganisms: Standard strains of enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E.coli* (EIEC) and *Shigella flexneri,* pre-tested with multiplex PCR were kindly provided by NAMRU-3, Cairo, Egypt. Ampicillin and kanamycin sensitive *E. coli* (standard K12 *E. coli* strain) was kindly obtained from Faculty of Pharmacy, Tanta University. A total of 147 *E. coli* isolates were used in the present study. These isolates were collected from food samples and clinical specimens in a previous study [4], where the antibiogram and antibiotic resistance profile of these isolates was also studied against 26 different members of antibiotics. All bacterial strains were stored and handled according to Aly *et al.* [4].

Genotypic Characterization of Isolated *E. coli* Strains: All isolated *E. coli* strains were subjected to molecular screening of commonly reported virulence genes. This characterization was performed through 5 schemes of multiplex PCR reactions (Table 1). Initially, 3 pure colonies of each *E. coli* strain were picked from MacConkey agar plates and suspended in 200 µl v/v PCR grade (mili Q) water. The bacterial suspension was then boiled for 5 min in thermostatic water bath (D3165-Kottermann, Germany) and reconstituted directly into ice bath until use. Finally, the suspension was centrifuged at 1400 g for 5 min and the supernatant was collected and used in the PCR reactions. All premiers and PCR reactions were designed and conducted according to Rappelli *et al.* [11]. All necessary positive controls (using standard *E. coli* strains) and negative controls (without any DNA addition) were conducted within each PCR set up.

Probability of Transfer And/or Acquisition of Antibiotic Resistance: The ability of transferring and/or acquiring antibiotic resistance characteristics among isolated *E. coli* strains was carried out using Ampicillin and/or Kanamycin-resistant *E. coli* isolates (isolates # 80, 83 and 117) as donor cells and ampicillin and kanamycin sensitive *E. coli* (standard K12 *E. coli* strain) as recipient cells as described by Uma *et al.* [12] with minor modification. Each of donor *E. coli* strain as well as recipient *E. coli* were allowed to grow to logarithmic phase on individual basis using nutrient broth in an incubator shaker (C25KC, Edisson, New Brunswick Scientific) at 37°C and 70 rpm for 18 h. For all strains the inoculum's size was initially adjusted using pre-determined correlation between viable count and optical density (600 nm) of each strain (data not shown).

The prepared bacterial suspensions were used to inoculate tested tubes filled with 5 ml nutrient broth and supplemented with 50 µg ampicillin /ml, 50 µg kanamycin /ml or mixture of both antibiotics at the same concentrations. Each set of test tubes was inoculated with donor *E. coli*, recipient *E. coli* or a mixture of both at a ratio of 1:1. The final inoculum's size within each tube was adjusted to 10^6 CFU /ml.

All tubes were then covered with screw caps and incubated at 37 °C and 70 rpm. Samples of 200 µl were aseptically withdrawn after 8, 16, 24 and 32 h. of incubation. For each sample the viable count was determined using 10 fold serial dilution techniques according to Sohhayeb and Sonbol [13] with minor modification. Where determination of the viable count was carried out using nutrient agar plates supplemented with 50µg ampicillin /ml, 50 µg kanamycin /ml or mixture of both antibiotics at the same concentrations according to the antibiotic(s) used in the corresponding tube.

Negative Stain Electron Microscopy (NSEM): Samples from different stages of probability of transfer and acquisition of antibiotic resistance genes test were fixed in 2.5% (v/v) glutaraldehyde in PBS (pH7.2) for 1.5 h., rinsed with PBS and then dehydrated through an ethanol series [14]. Samples were dried and gold-palladium coated. NSEM examinations were made on a JSM-840 SEM (JEOL Ltd., Tokyo, Japan).

RESULTS

Genotypic Characterization of Isolated *E. coli:* In general, out of the 147 *E. coli* isolates used in the present study 140 showed pathogenic characteristics. Among these 140 isolates 123 showed a PCR product at 623 bp only (Fig. 1A), which corresponds to the *UidA* gene (Table 1), therefore, these isolates were classified as non categorized pathogenic *E. coli* (Fig. 1).

Table 1: Sequences and predicted lengths of PCR products of multiplex PCR assays for the simultaneous identification of Enterotoxigenic, Enteropathogenic, Enteroinvasive, Enterohemorrhagic and Enteroaggregative *E. coli* according to Rappelli *et al*.[11]

Assay #	*Target gene*	*Oligonucleotide sequence(5' to 3')*	Expected Size of amplified product (bp)
1	*elt*	F: TCTCTATGTGCACACGGAGC	
		R: CCATACTGATTGCCGCAAT	322
	sta	F: TCTTTCCCCTCTTTTAGTCAGTC	
		R: CCAGCACAGGCAGGATTAC	170
	UidA	F: CCAAAAGCCAGACAGAGT	
		R: GCACAGCACATCAAAGAG	623
2	*eaeA*	F: TGATAAGCTGCAGTCGAATCC	
		R: CTGAACCAGATCGTAACGGC	229
	bfpA	F.CACCGTTACCGCAGGTGTGA	
		R.GTTGCCGCTTCAGCAGGAGT	450
3	*stx1*	F: GAAGAGTCCGTGGGATTACG	
		R: AGCGATGCAGCTATTAATAA	130
	stx2	F(*): GGGTACTGTGCCTGTTACTGG	
		R: GCTCTGGATGCATCTCGGT	510
	ial	F: CTGGTAGGTATGGTGAGG	
		R: CCAGGCCAACAATTATTTCC	320
4	*st1b*	F.ATTTTTCTTTCTGTATTGTCTT	
		R.CACCCGGTACAAGCAGGATT	190
5	*aggR*	F: CTAATTGTACAATCGATGTA	
		R: AGAGTCCATCTCTTTGATAAG	457
	AAprobe	F: CTGGCGAAAGACTGTATCAT	
		R: CAATGTATAGAAATCCGCTGTT	629

(*) This primer was modified from GGGTACTGTGTGCCTGTTACTGG according to Rappelli *et al*. [11] to be GGGTACTGTGCCTGTTACTGG according to the blasting with *stx2* gene using the NCBI database and Bioinformatics tool

Table 2: Classification of the selected *E. coli* isolates in the present study according to the number of detected virulence genes and corresponding antibiotic resistance pattern

Isolate #	Origin	Category	Detected V.F	V.F #	Antibiotic class to which isolates showed resistance [(a)]
51	Clinical	ETEC	*elt, sta, st1b, UidA*	4	Tetracyclines
52	Clinical	ETEC	*elt, sta, UidA*	3	Sensitive
80	Clinical	ETEC	*elt, st1b, UidA*	3	Beta-lactams, Tetracyclines, Sulfonamid+Trimethoprim
83	Clinical	ETEC	*elt, UidA*	2	Beta-lactams, Tetracyclines
11	Food	ETEC	*elt*	1	Beta-lactams, Tetracyclines, Sulfonamid+Trimethoprim
53	Clinical	EPEC	*eaeA,bfbA,UidA*	3	Tetracyclines
57	Clinical	EPEC	*eaeA, UidA*	2	Beta-lactams, Tetracyclines, Sulfonamid+Trimethoprim, Quinolones, Aminoglycosides, Macrolides, Polymyxins
76	Clinical	EPEC	*eaeA, UidA*	2	Beta-lactams, Tetracyclines, Sulfonamid+Trimethoprim, Quinolones, Aminoglycosides, Macrolides, Polymyxins
110	Clinical	EPEC	*eaeA, UidA*	2	Beta-lactams,Macrolides, Quinolones, Tetracyclines, Sulfonamid+Trimethoprim
118	Clinical	EPEC	*eaeA, UidA*	2	Beta-lactams
112	Clinical	EPEC	*eaeA, UidA*	2	Beta-lactams,Macrolides, Tetracyclines, Sulfonamid+Trimethoprim
50	Food	EPEC	*eaeA*	1	Sensitive
5	Clinical	EPEC	*eaeA*	1	Beta-lactams
54	Clinical	EHEC	*eaeA, stx1,stx2, UidA*	4	Sensitive
55	Clinical	EHEC	*eaeA, stx1,stx2, UidA*	4	Sensitive
105	Clinical	EHEC	*Stx1, UidA*	2	Beta-lactams,Macrolides, Aminoglycosides, Tetracyclines,
117	Clinical	EHEC	*Stx1, UidA*	2	Sensitive
13	Food	Commensal	No detected genes	0	Beta-lactams
40	Food	Commensal	No detected genes	0	Beta-lactams
43	Food	Commensal	No detected genes	0	Beta-lactams, Aminoglycosides, Polymyxins
44	Food	Commensal	No detected genes	0	Sensitive
45	Food	Commensal	No detected genes	0	Beta-lactams,Macrolides,
46	Food	Commensal	No detected genes	0	Beta-lactams, Polymyxins
47	Food	Commensal	No detected genes	0	Sensitive

(a) Data from Aly *et al.* [4]

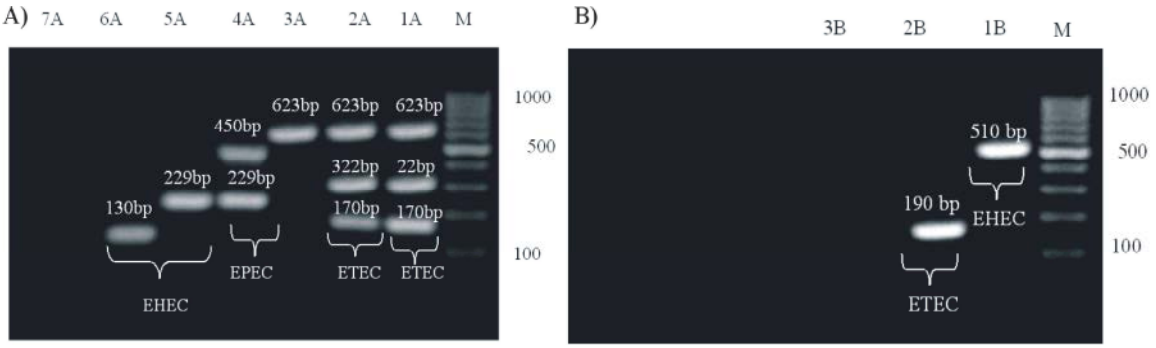

Fig. 1: The PCR products, which have been amplified by the sets of multiplex PCR reactions using DNA from isolated DEC strains. Lane M is 100-bp ladder, lane 1A and 2A (ETEC; *sta, elt and UidA*), lane 3A (Non-Categorized pathogenic *E. coli*; *UidA*), Lane 4A (Typical EPEC; *eaeA and bfpA*), lane 5A and 6A (EHEC; *eaeA and stx1*), lane 7A (negative control), lane 1B (EHEC; *stx2*), lane 2B (ETEC; *st1b*) and finally lane 3B (negative control).

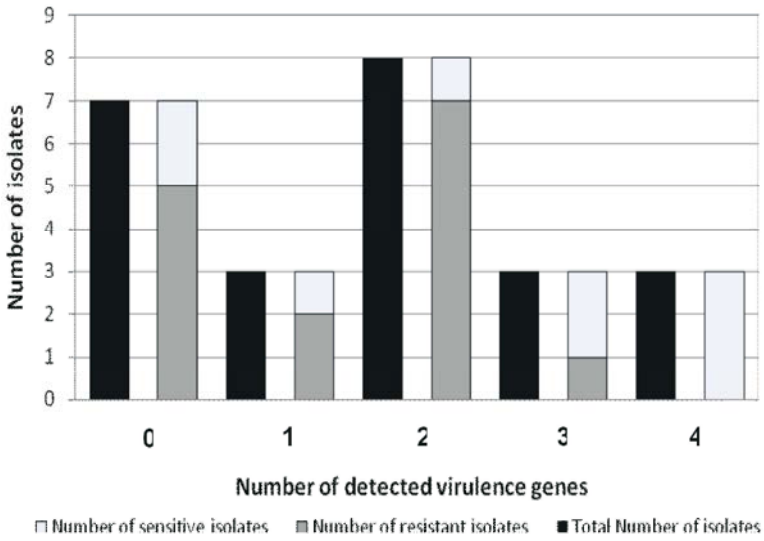

Fig. 2: The correlation between the degree of virulence and antibiotic resistance, where the recorded total number of *E. coli* isolates in the present study, classified according to the number of detected virulence genes (Black bars). Each class was further classified into antibiotics resistant isolates (gray bars) and antibiotic sensitive isolates (white bars)

A total of 17 isolates showed other PCR products with characteristics virulence genes of common DEC (Table 2). None of the tested virulence genes were detected in 7 isolates (all were collected from food samples), these isolates were considered to be non pathogenic or commensal *E. coli* (Table 2).

Among the characterized 17 DEC isolates, 8 strains (7 from clinical origin and one from food origin) showed PCR product at 229 bp (Fig. 1A). Besides, among these 8 isolates, 6 showed a second PCR product at 623 bp (Fig. 1A). These PCR products were correlated to *eaeA* and *UidA* genes respectively (Table 1). Additionally one of these 8 isolates showed another PCR product at 450 bp, which corresponds to *bfpA* gene (Fig. 1A and Table 1). Accordingly these 8 isolates were classified as EPEC (Table 2).

Moreover, 5 isolates (4 from clinical origin and one from food origin) showed PCR products at 623, 322, 170 and/or 190 bp (Fig. 1). These PCR products were corresponding to *UidA*, *elt*, *sta* and/or *st1b* respectively (Table 1) and accordingly theses isolates were classified as ETEC (Table 2). Four isolates showed PCR products at 623, 229, 130 and/or 510 (Fig. 1), which correspond to *UidA*, *eaeA, stx*1 and/or *stx*2 genes (Table) and accordingly these isolates were classified as EHEC. Interestingly, among these 4 EHEC 2 isolates had the 4 virulence genes (Table 2), while the other 2 isolates had only 2 virulence genes (*stx*1 and *UidA*) (Table 2).

Estimation of Possible Correlation Between Virulence Genes and Antibiotic Resistance in *E. coli* Isolates: Among the selected 24 isolates (17 isolates carrying virulence genes and 7 isolates free from virulence genes), 7 were sensitive to all tested antibiotics (Table 2). However, 6 isolates were resistant to only one class of the tested antibiotics (mainly Beta-lactams or Tetracyclines). Only 3 isolates were resistant to 2 classes of antibiotics (Table 2). The remaining 8 isolates showed resistance to 3 or more antibiotics classes (Table 2) and therefore, were classified as multi drug resistant *E. coli*. Comparatively, among these 24 isolates, 7 were free from all tested virulence genes and only one gene was detected in 3 isolates. The highest number isolates (8) were recorded for those had 2 virulence gens. Only three isolates had 3 virulence genes and the remaining three isolates had 4 virulence gens (Table 2).

Among the 7 isolates, which were free from all screened virulence genes, 5 were resistant to antibiotic and only 2 were sensitive (Fig. 2). Increasing the number of the virulence genes were accompanied with significant resistance, for instance, among the 8 isolates with 2 virulence genes, 7 isolates were resistant to antibiotics and only one was sensitive (Fig. 2). However, above 2 detected virulence genes the resistance pattern started to decline where among the three isolates, showed 3 virulence gene only one was resistant and even when the number of virulence genes was increased to 4 all isolates were sensitive (Fig. 2).

Probability of Transfer and/or Acquisition of Antibiotic Resistance Characteristics: The recorded viable counts of all donor cells were significantly increased after 8 h. of incubation and this number was even further increased after 16 h. (Table 3). However, after 24 h of incubation the number was too numerous to be counted at the highest dilution attempted (data not shown). On the other hand in all experiments when the recipient standard K12 *E. coli* strain was used alone as inoculum's no growth was detected (Table 3). When isolates 80 and 83 were used as inoculums alone or in combination with K12 *E. coli* no viable count was detected in presence of kanamycin or combination of ampicillin and kanamycin (Table 3). However, when isolate # 117 was used as inoculum alone or in combination with K12 *E. coli* viable counts more than 10^8 were recorded in the presence of these 2 antibiotics. Interestingly, when a combination of a donor *E. coli* and recipient *E. coli* was used as inoculums the recorded viable counts were almost 1-2 log cycles higher than those recorded when the donor cells were used alone under the same conditions and incubation time (Table 3).

NSEM monitoring of different growth stages of a combination of donor *E. coli* and K12 *E. coli* showed evidence of conjugation between these mated cells (Fig. 3). Where initially all cells were in a single arrangement (Fig. 3A). These cells started to gather and form initiatives of multichannel conjugation sites (Fig. 3B). Finally these multichannel conjugation sites appeared clearly between the mated cells (Fig. 3 C).

Table 3: The recorded aerobic viable counts within test tubes inoculated with 10^6 CFU /ml of either donor bacteria (isolate #80, 83 or 117) and/or K12 *E. coli* after different time intervals. All counts were determined using 10 fold serial dilution and cultivation on nutrient agar plates supplemented with 50 µg ampicillin l^{-1} and/or 50 µg kanamycin l^{-1}. All plates were incubated at 37°C. The recorded data represents mean ± SD

	Recorded viable count CFU/ml					
	8hr			16 hr		
Sample No.	AMP	K	AMP and K	AMP	K	AMP and K
Donor (80) (A+,K-)	(1±0.03) x10^{12}	0	0	(3±0.1) x10^{12}	0	0
Recipient (K12) (A-,K-)	0	0	0	0	0	0
Donor + recipient (A+,K-)	(7±0.2) x 10^{13}	0	0	(2.9±0.7) x10^{14}	0	0
Donor (83) (A+,K-)	(6±0.25)x10^{12}	0	0	(6.9±0.5) x10^{13}	0	0
Recipient(K12)	0	0	0	0	0	0
Donor + recipient (A+,K-)	(6±0.11)x10^{13}	0	0	(2.6±0.1) x10^{14}	0	0
Donor(117) (A+,K+)	(4±0.07) x10^8	(5.8±0.7) x10^8	(7.8±0.12) x10^9	(1±0.1)x10^{10}	(1.3±0.2)x10^{10}	(1.5±0.4) x10^{10}
Recipient(K12)	0	0	0	0	0	0
Donor + recipient A+,K+	(2.3±0.1) x10^9	(7±0.2) x10^9	(1.1±0.4)x10^{10}	(1.67±0.3) x10^{10}	(2±0.25)x10^{10}	(2.3±0.3) x 10^{10}

(A^+) = Resistant to Ampicillin, (A^-) = Sensitive to Ampicillin

(K^+) = Resistant to Kanamycin, (K^-) = Sensitive to Kanamycin

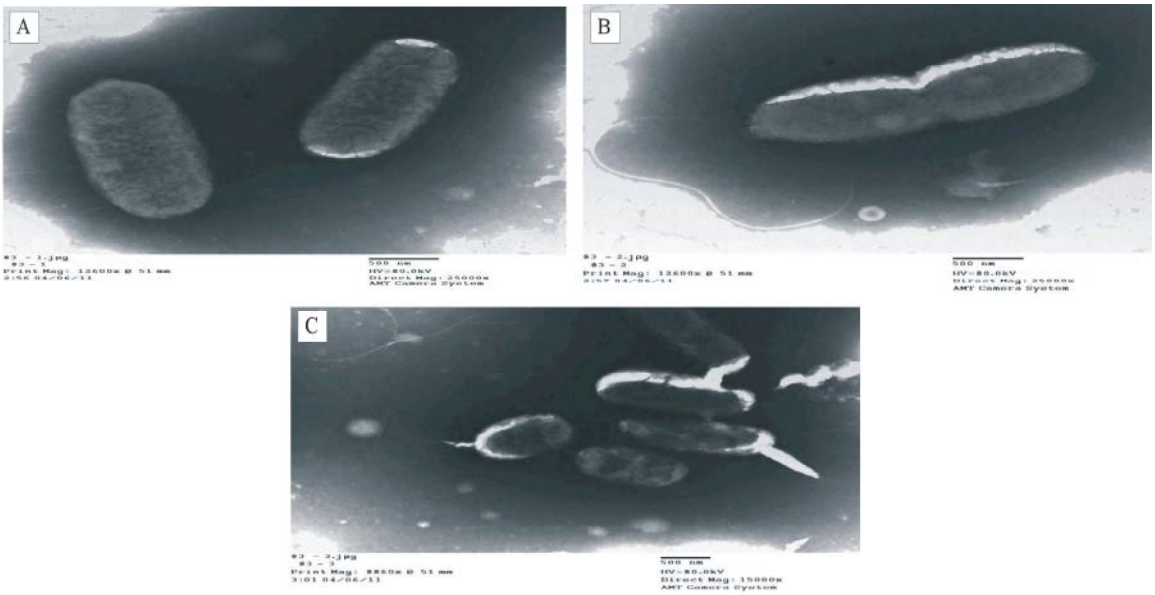

Fig. 3: NSEM of different growth stages of a combination between donor *E. coli* isolate and K12 *E. coli* strain cultivated in 1.5 ml Eppendorf tubes supplemented with nutrient broth medium. The bacteria showed single arrangement (A), intermediate stage of multichannel conjugation between 2 bacteria (B) and finally a full multichannel conjugation was appeared (C).

DISCUSSION

A total of 140 isolates showed the presence of at least one virulence gene and therefore, were classified as pathogenic *E. coli*. Among these pathogenic *E. coli*, 123 isolates had only *UidA* gene. This gene is coding for the adherence factor (glucuronidase) that is necessary to cause infection. Therefore, these isolates still retain its pathogenic trait although they were not belonged to any of the studied 5 categories of DEC [15]. Therefore, these isolates were classified as non categorized pathogenic *E. coli*. Yet the other 17 pathogenic *E. coli* isolates showed characteristic genes of DEC members. Totally, 8 isolates were classified as EPEC, among these isolates 7 were classified as atypical EPEC for not showing the *bfbA* gene and only one was typical EPEC where both *eaeA* and *bfbA* genes were detected [16]. Generally, the prevalence of atypical EPEC is more than typical ones as the *bfbA* gene is usually carried on the EAF (EPEC adherence factor) plasmid which can be easily cured by the microorganism in contrast to *eaeA* which present on chromosome [17].

Members of ETEC usually have 2 main kinds of virulence factors, fimbrial adhesions and enterotoxins, where 2 main categories of enterotoxins; heat-labile (LT) and heat-stable (ST) have been recognized so far. Additionally, 2 main types of these ST toxins were identified so far (*sta* and *st1b*) [18]. In the present study 5 isolates were classified as ETEC where all of these isolates had the LT gene. However, ST genes were detected only in 3 isolates where, one isolate had only *st1b*, another had *sta* gene alone and the third had both *st1b* and *sta*. Previously several studies have reported a similar inconsistent pattern of LT and/or ST production in ETEC [16].

Although, EHEC and EPEC share *eaeA* (the intimin structural gene), still the major virulence factors defining the characteristics of EHEC are *stx1* and *stx2* genes [19]. Two of those EHEC isolates had *eaeA* gene and both *stx1* and *stx2* genes, while the other 2 isolates had only *stx1* gene. Similarly, Rajendrana *et al.* [20], have reported that among the 8 isolated EHEC 4 had only *stx1* gene, 2 had *eaeA* gene with stx1 and the other 2 isolates had *eaeA* with *stx2* genes.

Conclusively, in present study the prevalence of DEC was in the following order EPEC, followed by ETEC then EHEC. Interestingly, none of isolated DEC strains were belonged to EAEC or EIEC. Although Rajendrana *et al*. [20] have reported that entero aggregative *E. coli* was the most prevalent DEC, the authors have reported a similar order of prevalence where EPEC were more prevalent than ETEC than EHEC among DEC isolated from children with diarrhea.

Bacterial virulence and the antibiotics resistance have been extensively studied on individual basis and the relationship between both is still lacking and was just estimated from epidemiological studies and data. Among these studies, Blazquez *et al*. [21], who have concluded that in bacteria increasing the number of virulence factors will increase in resistance till certain limit then the resistance decrease and the sensitivity increase. Similarly, José *et al*. [22] have reported that bacteria with intermediate levels of bacterial virulence have a greater probability of being exposed and developing resistance to antibiotics than less virulent and highly virulent organisms. This correlation was confirmed in the present study, where the antibiotic resistance was increased by increasing the detected virulence genes. Where, isolates had no virulence genes, one or 2 genes showed high degree of antibiotic resistance. However, this had a cutoff of 2 virulence genes as isolates with 3 virulence gens were more sensitive and those with 4 virulence genes were totally susceptible to all tested antibiotics.

This correlation could be explained that acquisition of either virulence and/or resistance gens should have a "direct" cost in bacterial fitness. Additionally, the genetic alternation may occur during acquiring the antibiotic resistance may lead to increased number of lethal mutations [23]. Hence, this reduction in the bacterial fitness or increased number of lethal mutations may affect the bacterial virulence by its reflection on the ability to invade or establish themselves within the host [23]. This also was supported by previously conducted Insilco bioinformatics studies that showed that *E. coli*, exhibit high plasticity caused by gene gain/loss via pathoadaptive mutations, genetic rearrangement and horizontal gene transfer [24].

Waste of humans and animals favours the development of antibiotics resistance in coliform and fecal coliform population especially *E. coli* [25]. Taking in to consideration that resistant bacterial strains spread in the environment through air and water and affect human health [26]. It was worth trying to investigate the probability of acquiring antimicrobial resistance characters between pathogenic resistant isolates and sensitive *E. coli*. Evidentially, K12 *E. coli* (sensitive *E. coli*) was able to acquire the ampicillin and kanamycin resistance characters from pathogenic resistant donor *E. coli*. Where, the recorded viable counts of combinations of donor *E. coli* and K12 *E. coli* were always 1-2 log cycles higher than that recorded for donor *E. coli* alone. Indeed, the resistance genes for kanamycin and ampicillin are highly transmittable and common in bacteria especially Gram negative [27, 28].

NSEM photos, captured at different growth stages of the mixture consisted of any of the 2 resistant isolates and K12 strain showed that the acquisition of the kanamycin and/or ampicillin resistance characters may be occurred through HGT by conjugation. Indeed, HGT is the most common mechanism of transferring and/or acquiring genes in bacteria [8]. Although Chiura *et al.* [29] have reported that HGT by transduction is the most common mechanism in *E. coli*, Uma *et al.* [12] have reported that HGT by conjugation still can occur in this genus.

The sequestered risk can be more explored by the hypothesis that the indigenous flora can become a reservoir of antibiotic resistance genes and these genes can be readily transferable to any invading pathogenic bacteria. This fear was shared by Schjfrring *et al*. [30], who have suggested that the Gram negative part of the flora has an increased prospect to obtain antibiotic resistance genes and might act as a reservoir and transfer the resistance gene further to pathogenic bacteria, which might lead to infections with limited treatment possibilities.

Possible correlation found between the virulence and antibiotic resistance in *E. coli* could be suggested. In most instances increasing the number of virulence gens was accompanied with increasing the antibiotic resistance until a cutoff of 2 virulence gens after that increasing the virulence gens decreased the resistance. Evidences showed that antibiotics resistance characters were acquired in K12 *E. coli* strain through HGT by conjugation though other possible mechanisms could not be excluded.

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