

Gut Commensal *Escherichia coli*, a High-Risk Reservoir of Transferable Plasmid-Mediated Antimicrobial Resistance Traits

Mahmoud Mohamed Tawfik^{1,2}, Aliaa Ali Elshamy², Kareem Talaat Mohamed³, Nagwan Galal El Menofy⁴

¹Department of Microbiology and Immunology, Faculty of Pharmacy (For Boys), Al-Azhar University, Cairo, 11751, Egypt; ²Department of Microbiology and Immunology, Faculty of Pharmacy, Heliopolis University, Cairo, 11785, Egypt; ³Department of Microbiology and Immunology, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 6th October City, Giza, 11787, Egypt; ⁴Department of Microbiology and Immunology, Faculty of Pharmacy (For Girls), Al-Azhar University, Cairo, 11751, Egypt

Correspondence: Mahmoud Mohamed Tawfik, Department of Microbiology and Immunology, Faculty of Pharmacy (For Boys), Al-Azhar University, 1 El-Mokhayam El-Daem Street, Nasr City, Cairo, 11751, Egypt, Tel +20 1157336676, Fax +20 238371543, Email mahmoud_tawfik@azhar.edu.eg

Background: *Escherichia coli* (*E. coli*), the main human gut microorganism, is one of the evolved superbugs because of acquiring antimicrobial resistance (AMR) determinants via horizontal gene transfer (HGT).

Purpose: This study aimed to screen isolates of gut commensal *E. coli* from healthy adult individuals for antimicrobial susceptibility and plasmid-mediated AMR encoding genes.

Methods: Gut commensal *E. coli* bacteria were isolated from fecal samples that were taken from healthy adult individuals and investigated phenotypically for their antimicrobial susceptibility against diverse classes of antimicrobials using the Kirby Bauer disc method. PCR-based molecular assays were carried out to detect diverse plasmid-carried AMR encoding genes and virulence genes of different *E. coli* pathotypes (*eaeA*, *stx*, *ipaH*, *est*, *elt*, *aggR* and *pCVD432*). The examined AMR genes were β -lactam resistance encoding genes (*bla*_{CTX-M1}, *bla*_{TEM}, *bla*_{CMY-2}), tetracycline resistance encoding genes (*tetA*, *tetB*), sulfonamides resistance encoding genes (*sulI*, *sulII*), aminoglycoside resistance encoding genes (*aac(3)-II*, *aac(6')-Ib-cr*) and quinolones resistance encoding genes (*qnrA*, *qnrB*, *qnrS*).

Results: PCR results revealed the absence of pathotypes genes in 56 isolates that were considered gut commensal isolates. *E. coli* isolates showed high resistance rates against tested antimicrobial agents belonging to both β -lactams and sulfonamides (42/56, 75%) followed by quinolones (35/56, 62.5%), tetracyclines (31/56, 55.4%), while the lowest resistance rate was to aminoglycosides (24/56, 42.9%). Antimicrobial susceptibility profiles revealed that 64.3% of isolates were multidrug-resistant (MDR). High prevalence frequencies of plasmid-carried AMR genes were detected including *bla*_{TEM} (64%) *sulI* (60.7%), *qnrA* (51.8%), *aac(3)-II* (37.5%), and *tetA* (46.4%). All isolates harbored more than one gene with the most frequent genetic profile among isolates was *bla*_{TEM}-*bla*_{CTX-M1}-like-*qnrA*-*qnrB*-*tetA*-*sulI*.

Conclusion: Results are significant in the evaluation of plasmid-carried AMR genes in the human gut commensal *E. coli*, suggesting a potential human health risk and the necessity of strict regulation of the use of antibiotics in Egypt. Commensal *E. coli* bacteria may constitute a potential reservoir of AMR genes that can be transferred to other bacterial species.

Keywords: gut microorganisms, antibiotic resistance, plasmid, MDR, resistance genes

Introduction

In recent years, antimicrobial resistance (AMR) has become a global threat to public health.^{1,2} The urgent AMR crisis and transmission of multidrug-resistant (MDR) bacterial pathogens are major causes of high mortality and morbidity rates worldwide.³ While the reasons for the high rates of AMR, particularly in developing countries, still require further research.⁴ Many studies reported the extensive dissemination of AMR among Gram-positive and Gram-negative bacteria in Egypt, which requires continuous monitoring of antimicrobial resistance profiles, efficient diagnosis and implementation of effective antibiotic stewardship programs.^{5–7} AMR encoding genes are the main mechanisms for developing resistance to different classes of antimicrobials among bacteria. These AMR resistance

encoding genes may be carried on the bacterial chromosome or located on mobile genetic elements acquired by bacteria such as plasmids.^{8,9}

Commensal microorganisms or microbiota, mainly gut commensal bacteria, can act as a reservoir of AMR genes. Hence, these resistance genes can be transferred from these commensal bacteria to pathogenic bacterial strains.¹⁰ Particularly, gut commensal bacteria that are found in huge numbers in the gut are at risk of acquiring genes encoding for AMR traits owing to high exposure to oral antimicrobial therapy, in addition to some parenteral preparations.¹¹ The antimicrobial susceptible gut commensals and/or pathogens will be eradicated. However, the resistant microorganisms of both groups will survive and become predominant in a specified site.^{8,9} Additionally, some antibiotics are used in sub-therapeutic levels as animal feed additives to promote growth and prevent infections.^{12,13} Frequent exposure and misuse of antibiotics in both humans and animals has upsurged the emergence and spread of AMR.^{12,14}

Escherichia coli is a bacterium that has an importance in the microbiological world, it has a dual role as a member of the gut microbiota the first bacterial species colonizing the gut.¹⁵ In addition, it is one of the most common human and animal pathogens that can cause intestinal and extra-intestinal infections.^{2,8} Commensal *E. coli* is normally residing in the gut lumen and rarely causes disease. However, in case of host impaired immunity or breached gastrointestinal barriers, commensal *E. coli* can cause opportunistic infections in its host such as urinary tract infections.¹⁶ Though, these *E. coli* strains can acquire AMR and virulence traits encoding genes through DNA horizontal transfer of mobile genetic elements (MGEs) including plasmids, transposons and pathogenicity islands and bacteriophages which confer bacteria to cause resistant infectious diseases.¹⁷ Furthermore, commensal *E. coli* might act as a reservoir of MGEs, such as transposons and plasmids, that are carrying AMR and/or virulence traits encoding genes.¹⁵ Thus, remarkably, commensal *E. coli* can act as a donor and/or a recipient of AMR genes in the enterobacterial gene pool; they acquire and pass resistance genes from and to other bacteria by HGT.¹⁸ Conjugation, via the assembly of conjugative pili, is identified as the most common mechanism for the transmission of plasmids carrying AMR genes especially those encoding extended-spectrum beta-lactamases and carbapenemases.^{19,20} Significantly, the gut bacteria can conceal more than one thousand different AMR genes which will be easily transmitted via the fecal-oral route in both humans and animals.²¹

Regarding infections, *E. coli* is the most common cause of urinary tract infections (UTIs), although it can infect other anatomical sites in the human body in all age groups.^{15,22} It can cause meningitis, septicemia, gastrointestinal infections, bloodstream infections, appendicitis, endocarditis, pneumonia and skin abscesses.^{2,22} In women, gut commensal *E. coli*, through the ascending route, is the main cause of UTIs due to the short distance between the urethral meatus and anus; that may extend to the bladder wall and kidney.²³

Since the emergence of AMR and the issue of resistant commensal gut bacteria in healthy people have yet not been addressed in Egypt as far as we know, the present study was planned to determine the antimicrobial susceptibility profile of gut commensal *E. coli* isolates from healthy adult individuals. In addition, the study aimed to investigate the harboring of plasmid-carried AMR genes encoding for resistance to diverse classes of antimicrobials by these commensal gut *E. coli* isolates.

Materials and Methods

Study Population and Fecal Samples

The present study included 72 non-duplicate fecal samples collected from healthy Egyptian adults. These healthy individuals were essentially selected as a must who are in good health and do not suffer from any gastric disorders or other diseases and did not take any antibiotics at least three months before collection of fecal samples. The fecal samples were collected in sterile dedicated containers sealed in stretch film, and then transported to the microbiology laboratory within one hour and kept at 4°C until processing. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Heliopolis University (Ethics Approval Code: HU.REC.H.5-2021).

Fecal Culture and Identification of Gut Commensal *E. coli*

The fecal samples were processed and cultured on the same day of collection following the procedures of Stanley et al.²⁴ The feces was mixed with sterile normal saline and the fecal suspension was cultured on MacConkey agar medium, then the agar plates were incubated overnight at 37°C. The lactose fermenting colonies with a typical appearance suggestive of *E. coli* bacteria were picked and subjected to further identification. *E. coli* isolates were identified using standard microbiological laboratory methods including cultural characteristics on the selective medium; eosin methylene blue (EMB) (Oxoid® Limited, Basingstoke, UK), Gram-staining and biochemical tests according to the previously mentioned identification scheme.²⁵ Identification was confirmed by Vitek 2 automated system using VITEK® 2 GN panel according to the manufacturer's guidelines (bioMe'rieux, Marcy l'E'toile, France). One *E. coli* isolate was recovered from each one of the non-repetitive 72 fecal samples that were obtained from each one of the included 72 healthy individuals, who did not take antibiotics during the last three months before collection of the fecal sample. The commensal *E. coli* was identified by the absence of *E. coli* pathotypes genes (mentioned in Table 1) that were examined by PCR. The isolates were preserved at -20°C in glycerol stock media until examined for their antimicrobial susceptibility patterns and PCR studies.

Table 1 Target Genes, Sequences of PCR Oligonucleotide Primers and Expected PCR Product Size

Target Gene	Sequence (5' – 3')	Amplicon Size (bp)	Ta/Extension Time	Source
Resistance to beta-lactam antibiotics (β-lactamases encoding genes)				
<i>bla</i> _{TEM}	F: ATAAATTCCTTGAAGAC R: TTACCAATGCTTAATCA	1075	42°C/1 min	[66]
<i>bla</i> _{CTX-M1-like genes}	F: TTAATTCGTCTCTTCCAGA R: CAGCGCTTTTGCCGTCTAAG	1042	45°C/1 min	
<i>bla</i> _{CMY-like genes}	F: ATGATGAAAAAATCGATATG R: TTATTGCAGTTTTTCAAGAATG	1146	45°C/1 min	
Resistance to aminoglycoside antibiotics				
<i>aac(3)-II</i>	F: TGAAACGCTGACGGAGCCTC R: GTCGAACAGGTAGCACTGAG	369	55°C/30 s	[66]
<i>aac(6')-Ib-cr</i>	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGT	482	55°C/30 s	[67]
Resistance to quinolones				
<i>qnrA</i>	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	52°C/40 s	[67]
<i>qnrB</i>	F: GATCGTGAAAGCCAGAAAGG R: ATGAGCAACGATGCCTGGTA	476		
<i>qnrS</i>	F: GCAAGTTCATTGAACAGGGT R: TCTAAACCGTCGAGTTCGGCG	428		
Resistance to tetracyclines				
<i>tetA</i>	F: GCTACATCCTGCTTGCCT R: CATAGATCGCCGTGAAGA	210	52°C/30 s	[68]
<i>tetB</i>	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	600	52°C/40 s	

(Continued)

Table 1 (Continued).

Target Gene	Sequence (5' – 3')	Amplicon Size (bp)	Ta/Extension Time	Source
Resistance to sulfonamides				
<i>sulI</i>	F: TGGTGACGGTGTTCGGCATT R: GCGAAGGTTTCCGAGAAGGTG	790	56°C/50 s	[66]
<i>sulII</i>	F: CGGCATCGTCAACATAACCT R: TGTGCGGATGAAGTCAGCTC	721	56°C/50 s	
Type of <i>E. coli</i> pathotype (virulence-associated genes in <i>E. coli</i>)				
EPEC: <i>eaeA</i>	F: AAACAGGTGAACTGTTGCC R: CTCTGCAGATTAACCCTCTGC	454	52°C/30 s	[7]
EAEC: <i>aggR</i>	F: CTAATTGTACAATCGATGTA R: ATGAAGTAATTCTTGAAT	308	42°C/30 s	
EAEC: <i>pCVD432</i>	F: CTGGCGAAAGACTGTATCAT R: CAATGTATAGAAATCCGCTGTT	630	55°C/40 s	
STEC/EHEC: <i>stx</i>	F: GAGCGAAATAATTTATATGTG R: TGATGATGGCAATTCAGTAT	518	42°C/30 s	[69]
ETEC: <i>est</i>	F: TTAATAGCACCCGGTACAAGCAGG R: CCTGACTCTTCAAAAGAGAAAATTAC	147	52°C/30 s	
ETEC: <i>elt</i>	F: TCTCTATGTGCATACGGAGC R: CCATACTGATTGCCGCAAT	322	52°C/30 s	
EIEC: <i>ipaH</i>	F: GTTCCTTGACCGCCTTCCGATACCGTC R: GCCGGTCAGCCACCCTCTGAGAGTAC	619	58°C/40 s	

Abbreviations: PCR, polymerase chain reaction; *E. coli*, *Escherichia coli*; EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; STEC, Shiga toxin-producing *E. coli*; ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EHEC, enterohemorrhagic *E. coli*; Ta, annealing temperature; bp, base pair.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility phenotypes of *E. coli* isolates were determined using the Kirby-Bauer disc diffusion method²⁶ on Mueller-Hinton agar (MHA) (Oxoid® Limited, Basingstoke, UK) following the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines.²⁷ The following 14 antimicrobials, representing different classes of antimicrobial agents, were tested: ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), ceftriaxone (30 µg), cefoxitin (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), amikacin (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The antimicrobial discs were the product of Oxoid® Limited, Basingstoke, UK. These antimicrobials were selected considering the target genes to be investigated and that are of clinical importance in treating bacterial infections in humans in Egypt. The inhibition zones formed around the discs, measured in mm, were interpreted as susceptible (S) or resistant (R) to a particular antimicrobial agent according to CLSI breakpoints. According to Magiorakos et al, *E. coli* isolate was considered as MDR if it is non-susceptible to three or more different antimicrobial classes.²⁸ The standard strain *E. coli* ATCC 25922 was used as the quality control in the antimicrobial susceptibility testing. The correlation between AMR phenotypic and genotypic patterns of *E. coli* isolates was assessed.

Identification of ESBLs *E. coli* Phenotypes

E. coli isolates were screened for potential Extended-Spectrum β-Lactamases (ESBLs) production. *E. coli* isolates were considered as ESBLs-producer according to CLSI guidelines of being resistant to penicillins, third- and fourth-generation cephalosporins and aztreonam. These isolates were selected for confirmation of ESBLs production according to the CLSI

combination disc confirmatory test, or the disc diffusion clavulanate inhibition test, using both cefotaxime and ceftazidime alone and in combination with clavulanate.²⁷ The antimicrobial discs used for this test were ceftazidime (30 µg) and ceftazidime-clavulanate (30 µg/10 µg), cefotaxime (30 µg) and cefotaxime-clavulanate (30 µg/10 µg). The test was carried out using the standard disc diffusion procedure on MHA. The isolate was considered ESBLs-producer if the inhibition zone diameter of both antimicrobial agents' combination discs was ≥ 5 mm than that of the ceftazidime or cefotaxime disc alone. *E. coli* ATCC 25922 was used as a negative control for the evaluation of ESBLs production. The accepted quality control limit is ≤ 2 -mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.

PCR-Based Molecular Experiments

DNA Extraction and PCR Oligonucleotide Primers

Total crude DNA was extracted from all tested isolates using the boiling method. Bacterial cell suspensions in 50 µL of molecular biology-grade water were subjected to boiling at 100°C for 10 min, followed by removal of cellular debris by centrifugation at 15,000 $\times g$ for 30s. The supernatant was collected and stored at -20°C for use as template DNA for PCR. Aliquots of 2 µL of template DNA were used for PCR assays to identify *E. coli* pathotypes. For detection of plasmid-carried AMR genes, plasmid DNA was extracted from *E. coli* isolates using Gene JET™ Plasmid Miniprep Kit (Thermo Scientific, Waltham, USA) according to the manufacturers' instructions.²⁹ The extraction of plasmid DNA from all isolates was analyzed as the extracted DNA solution was subjected to electrophoresis on a 0.7% agarose gel (Bioline, UK) against 1 kb DNA ladder marker (TianGen, China). Extracted DNA was collected and stored in small aliquots at -20°C and used as a template in PCR experiments. The sequences of the previously published PCR oligonucleotide primers used in this study, synthesized by Invitrogen (UK), are listed in Table 1. The lyophilized powder of each primer was reconstituted using nuclease-free water to achieve a concentration of 100 pmol/µL and then was adjusted to the working concentration of 10 pmol/µL.

PCR Assays for Detection of Antimicrobial Resistance Genes and Identification of *E. coli* Pathotypes

E. coli isolates were examined by PCR for selected plasmid-carried AMR genes (available at GenBank database) encoding for resistance to diverse classes of antimicrobial drugs and different *E. coli* pathotypes genes (Table 1). PCR reaction mixtures were prepared in total volumes of 20 µL. Each reaction contained 2 µL of template DNA, 1 µL (equivalent to 10 pmol concentration) of each primer and 10 µL of GoTaq® Green Master 2 \times Ready Mix (Promega, Madison, USA), then the volume was completed to 20 µL by adding 6 µL of nuclease-free water. The PCR amplification programs included initial denaturation for 5 min at 95°C, then 35 cycles of denaturing at 95°C for 30 seconds, annealing for 30 seconds and extension at 72°C, followed by a final extension at 72°C for 7 min. The appropriate annealing temperature for each pair of primers and the time for the extension step for each PCR amplicon are mentioned in Table 1.

DNA fragments of PCR products were detected using TAE agarose gel (0.8%) (Bioline, London, UK) electrophoresis in 1 \times TAE buffer containing ethidium bromide for DNA visualization on a UV light source. A suitable GeneRuler DNA molecular weight marker (Thermo Scientific, USA) was used for sizing the PCR products.

Data Analysis

Data were analyzed using Eviews 8 software. Data were presented in tables as relative percentages and frequencies outputs for resistance patterns to different antimicrobial agents and gene variables.

Results

Identification of Gut Commensal *E. coli* Isolates

In this study, 72 non-repetitive *E. coli* isolates were recovered from the 72 fecal samples of healthy individuals (one *E. coli* isolate from each fecal sample that was taken from each individual). Out of these 72 *E. coli* isolates, 56 isolates (56/72, 77.8%) were identified as gut commensals. These 56 *E. coli* isolates showed the typical phenotypic characteristics of *E. coli* including the cultural characteristics, on McConkey and EMB media and specific biochemical reactions, as well as VITEK® 2 automated system. In addition, PCR results revealed the absence of *E. coli* pathotypes virulence genes

(*eeA*, *stx*, *ipaH*, *est*, *elt*, *aggR* and *pCVD432*) in these 56 isolates. Twelve *E. coli* isolates other than these 56 isolates were excluded due to the presence of any of these pathotypes virulence genes.^{30–32}

Antimicrobial Susceptibility Patterns and Frequency of MDR Isolates

The antimicrobial susceptibility pattern of the 56 *E. coli* gut commensal isolates against 14 antimicrobial agents is presented in Table 2. Among these isolates, 78.6% (44/56) of isolates showed resistance to all of the tested antimicrobial agents, while 21.4% (12/56) showed no resistance to any one of them (ie, sensitive to all tested antimicrobials). Generally, *E. coli* isolates showed high resistance rates against tested antimicrobial agents. According to the antimicrobial classes, the highest resistance frequency was against tested antimicrobial agents belonging to both β -lactams and sulfonamides (42/56, 75%) followed by quinolones (35/56, 62.5%), tetracyclines (31/56, 55.4%), while the lowest resistance rate was to aminoglycosides (24/56, 42.9%).

In detail, the highest resistance rate of 75% was recorded against each ampicillin (indicator for amoxicillin resistance as well) and trimethoprim/sulfamethoxazole, followed by the resistance to ceftriaxone (73.2%), then each cefuroxime, ceftazidime and aztreonam (71.4%) and cefepime (69.6%). There was also a significant frequency of resistance to amoxicillin-clavulanate (66.1%), tested fluoroquinolones (62.5%), tetracycline (55.4%) and cefoxitin (64%). Lower resistance rates were determined to amikacin (32.1%) and gentamicin (42.9%). Of the 44 isolates that showed resistance to antimicrobial agents, 81.8% (36/44) of these isolates or 64.3% (36/56) of total isolates included in this study were MDR which were resistant to at least three antimicrobials belonging to three different antimicrobial classes.

Distribution of ESBLs-Producing *E. coli* Phenotypes

A total of 71.4% (40/56) of *E. coli* isolates were considered as potential ESBLs producers according to CLSI guidelines of being resistant to ceftriaxone, ceftazidime, cefepime or aztreonam. However, 67.9% (38/56) isolates showed positive results of the confirmatory combination disc method for detection of ESBL production based on cefotaxime and ceftazidime susceptibility alone or in combination with clavulanate.

Table 2 Distribution of Antimicrobial Resistance Among Gut Commensal *E. coli* Isolates

Antimicrobial Agent	Sensitive No. (%) ^a	Resistant No. (%) ^a
Ampicillin	14 (25)	42 (75)
Amoxicillin-clavulanate	19 (33.9)	37 (66.1)
Cefoxitin	26 (46.4)	30 (53.6)
Ceftriaxone	15 (26.8)	41 (73.2)
Cefuroxime	16 (28.6)	40 (71.4)
Ceftazidime	16 (28.6)	40 (71.4)
Cefepime	17 (30.4)	39 (69.6)
Aztreonam	16 (28.6)	40 (71.4)
Ciprofloxacin	21 (37.5)	35 (62.5)
Levofloxacin	21 (37.5)	35 (62.5)
Gentamicin	32 (57.1)	24 (42.9)
Amikacin	38 (67.9)	18 (32.1)
Tetracycline	25 (44.6)	31 (55.4)
Trimethoprim/sulfamethoxazole	14 (25)	42 (75)

Note: ^aPercentage correlated to the total number of commensal isolates (n = 56).

Abbreviation: *E. coli*, *Escherichia coli*.

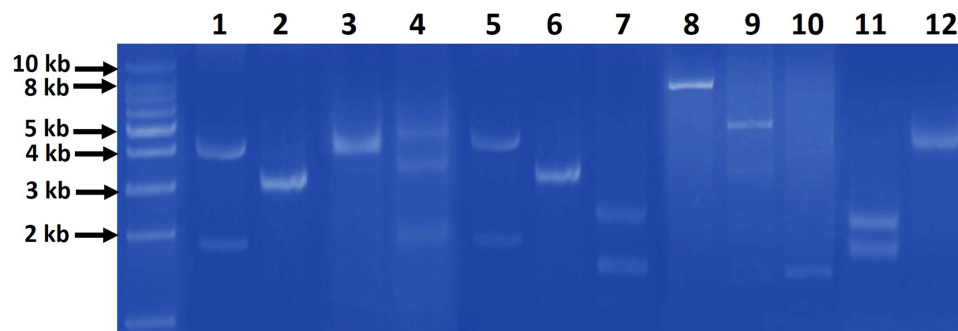


Figure 1 Representative agarose gel (0.7%) electrophoresis of the different patterns of extracted plasmids from gut commensal *E. coli* isolates. The patterns comprised of one band (lanes 2, 3, 6, 8, 10, 12), two bands (lanes 1, 5, 7, 9, 11) and three bands (lane 4) of different sizes. First most left lane, 1 kb DNA molecular weight marker.

Plasmid Separation and Frequencies of Target Plasmid-Carried Antimicrobial Resistance Genes by PCR Assays

Agarose gel electrophoresis of the plasmid DNA extracts revealed that there were detectable plasmids in 44 (78.6%) isolates while 12 isolates (21.4%) had no plasmids. Isolated intact plasmids showed different patterns among isolates which comprised from one to three bands of different sizes (Figure 1).

Overall, based on PCR assays targeting genes encoding resistance to different antimicrobial agents/classes, the most widespread genes among isolates were *bla*_{TEM} and *sulI* with frequencies of 66.1% and 60.7%, respectively, followed by *qnrA* with a frequency of 51.8% (Table 3). In addition, PCR results revealed the absence of *E. coli* pathotypes virulence genes (*eaeA*, *stx*, *ipaH*, *est*, *elt*, *aggR* and *pCVD432*) in these 56 isolates.

The distribution of AMR genes with corresponding AMR phenotypes among *E. coli* isolates is also demonstrated in Table 3. This matching revealed similar phenotypic susceptibility patterns of the isolates versus coexisting quinolones resistance genes, tetracyclines resistance genes and sulfonamides resistance genes. While there was a different distribution pattern for β -lactams resistance genes and resistance patterns to amoxicillin-clavulanate, aztreonam, ceftazidime, cefepime and between aminoglycoside resistance genes and amikacin (Table 3 and Figure 2).

The 44 isolates that showed resistance to any of the tested antimicrobials (44/56, 78.6%) harbored more than one AMR encoding gene while 12 isolates showed no carrying of any resistance genes as they showed no harboring of plasmids and exhibited full phenotypic sensitivity pattern to all tested antimicrobial agents. The most frequent genetic profile among isolates was *bla*_{TEM}-*bla*_{CTX-M1-like}-*qnrA*-*qnrB*-*tetA*-*sulI* in 21.4% of isolates, followed by *bla*_{TEM}-*aac*(3)-*II*-*aac*(6')-*Ib*-*cr*-*qnrA*-*tetA*-*tetB*-*sulI* that was found in 14.3% of isolates (Table 4).

Discussion

Resistance to antimicrobial drugs is largely believed to be a consequence of human activities, for instance, the extensive and/or misuse of antibiotics. While many studies revealed the presence of significant numbers of AMR encoding genes within the genomes of human bacterial flora as well as environmental bacteria.^{1,10,15,33,34} In particular, the resistance of *E. coli* to diverse antimicrobial classes owing to either acquired or extrinsic mechanisms is already a major public health problem.¹ Gut commensal *E. coli* is one of the most important reservoirs of AMR genes leading to treatment failure of infections in both human and veterinary medicine.³⁵ Presently, it is important to determine the bacterial AMR patterns and virulence to reduce the risk of complications and/or avoid therapy failure of infections especially those caused by MDR strains.³⁶ Hence, we aimed in the current study to investigate gut commensal *E. coli* isolates from healthy individuals for antimicrobial susceptibility and plasmid-mediated AMR genes.

Here, we report a high rate (64.3%) of MDR *E. coli* bacteria isolated from the feces of clinically healthy individuals. These *E. coli* bacteria carry various AMR genes without the existence of pathotype virulence-associated genes including EPEC: *eaeA*, EAEC: *aggR*, EAEC: *pCVD432*, STEC/EHEC: *stx*, ETEC: *est*, ETEC: *elt* and EIEC: *ipaH*. In most cases, a high rate of AMR is associated, either directly or indirectly, with decreased virulence and fitness.³⁷ However, in other

Table 3 Distribution of AMR Genes with Corresponding AMR Phenotypes Detected in *E. coli* Isolates

Target Genes Encoding Diverse Antimicrobial Classes	Prevalence of Target Genes Among <i>E. coli</i> Isolates (n = 56) No. (%) ^a	AMR Testing of <i>E. coli</i> Isolates with Corresponding Resistance Genes No. (%) ^a							
β-Lactams Resistance Genes		Ampicillin	Amoxicillin-Clavulanate	Aztreonam	Cefoxitin	Ceftriaxone	Cefuroxime	Ceftazidime	Cefepime
<i>bla</i> _{TEM}	37 (66.1)	37 (100)	33 (89.1)	35 (94.5)	26 (70.2)	36 (97.3)	35 (94.5)	35 (94.5)	34 (91.9)
<i>bla</i> _{CTX-M1-like} genes	24 (42.8)	24 (100)	19 (79.1)	22 (91.6)	17 (70.8)	24 (100)	24 (100)	24 (100)	24 (100)
<i>bla</i> _{CMY-like} genes	6 (10.7)	6 (100)	6 (100)	6 (100)	4 (66.6)	6 (100)	6 (100)	6 (100)	5 (83.3)
Aminoglycoside Resistance Genes		Gentamicin				Amikacin			
<i>aac(3)-II</i>	24 (42.9)	24 (100)				18 (75)			
<i>aac(6')-Ib-cr</i>	14 (25)	14 (100)				12(85.7)			
Quinolones Resistance Genes		Ciprofloxacin				Levofloxacin			
<i>qnrA</i>	31 (55.4)	31 (100)				31 (100)			
<i>qnrB</i>	17 (30.4)	16 (94.1)				16 (94.1)			
<i>qnrS</i>	0 (0)	0 (0)				0 (0)			
<i>aac(6)-Ib-cr</i>	14 (25)	13 (92.8)				13 (92.8)			
Tetracyclines Resistance Genes		Tetracycline							
<i>tetA</i>	27 (48.2)	27 (100)							
<i>tetB</i>	14 (25)	14 (100)							
Sulfonamides Resistance Genes		Trimethoprim/Sulfamethoxazole							
<i>sull</i>	34 (60.7)	34 (100)							
<i>sullI</i>	18 (32.1)	18 (100)							

Note: ^aPercentage correlated to the total number of commensal isolates (n = 56).

Abbreviations: *E. coli*, *Escherichia coli*; AMR, antimicrobial resistance.

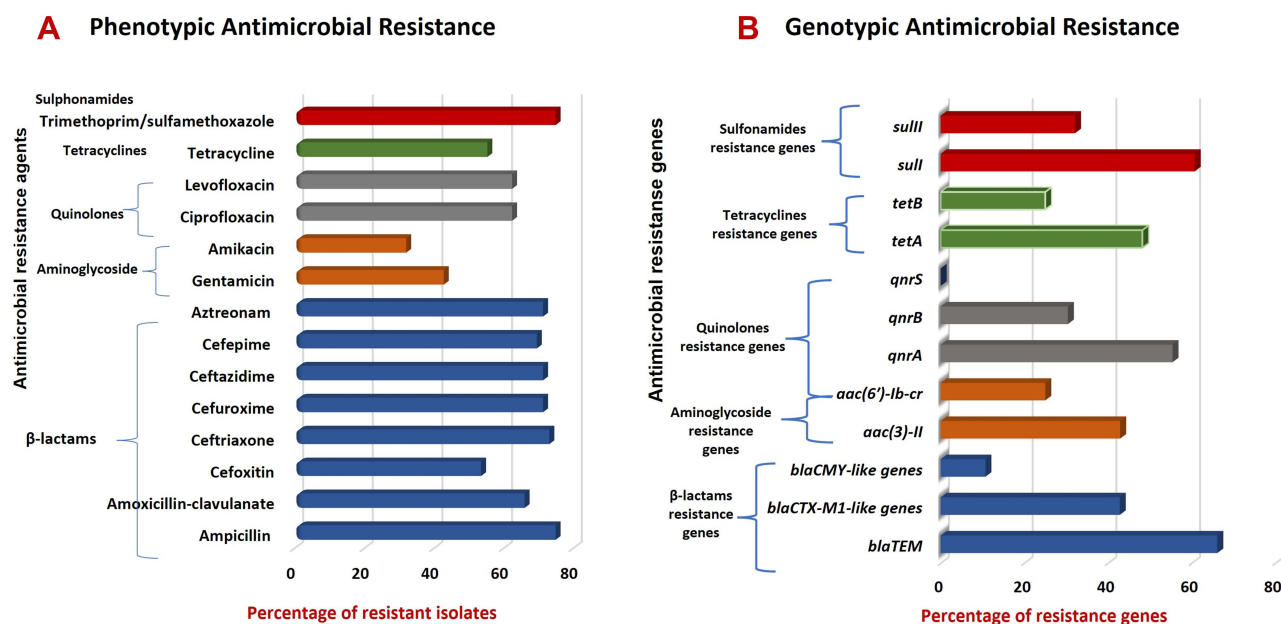


Figure 2 Prevalence of AMR-phenotypes and associated genes in commensal *E. coli* isolates. (A) AMR phenotypes among *E. coli* isolates; (B) AMR-associated genes harbored by *E. coli* resistant phenotypes.

studies, highly virulent microorganisms showed high resistance profiles as well.^{12,38} Although, it is increasingly evident that the presence of both virulence and AMR traits is likely of greater benefit to the microorganism.³⁷ Virulence factors are necessary to overcome host defense mechanisms, and the development of AMR is essential to enable pathogenic bacteria to overcome antimicrobial drugs and to adapt to and survive in competitive and demanding environments.³⁷ Notably, although AMR is not itself a virulence factor, it is a key factor in developing infections in certain situations.

Table 4 Genotypic Profiles of AMR Genes Among *E. coli* Isolates

Genotypic Profile	Frequency ^a
<i>bla</i> _{TEM} - <i>bla</i> _{CTX-M1-like} - <i>qnrA</i> - <i>qnrB</i> - <i>tetA</i> - <i>sulI</i>	12/56, 21.4%
<i>bla</i> _{TEM} - <i>aac</i> (3)- <i>II</i> - <i>aac</i> (6')- <i>Ib-cr</i> - <i>qnrA</i> - <i>tetA</i> - <i>tetB</i> - <i>sulI</i>	8/56, 14.3%
<i>bla</i> _{CTX-M1-like} - <i>aac</i> (3)- <i>II</i> - <i>qnrA</i> - <i>tetB</i> - <i>sulI</i> - <i>sulII</i>	4/56, 7.1%
<i>bla</i> _{TEM} - <i>bla</i> _{CTX-M1-like} - <i>aac</i> (3)- <i>II</i> - <i>aac</i> (6')- <i>Ib-cr</i> - <i>qnrA</i> - <i>sulI</i>	4/56, 7.1%
<i>bla</i> _{TEM} - <i>qnrA</i> - <i>aac</i> (3)- <i>II</i> - <i>tetA</i> - <i>sulII</i>	3/56, 5.4%
<i>bla</i> _{TEM} - <i>bla</i> _{CMY-like} - <i>qnrB</i> - <i>aac</i> (3)- <i>II</i> - <i>sulI</i> - <i>sulII</i>	3/56, 5.4%
<i>bla</i> _{TEM} - <i>bla</i> _{CMY-like} - <i>sulI</i> - <i>sulII</i>	3/56, 5.4%
<i>bla</i> _{TEM} - <i>bla</i> _{CTX-M1-like} - <i>sulI</i>	2/56, 3.6%
<i>tetA</i> - <i>tetB</i> - <i>sulII</i>	2/56, 3.6%
<i>bla</i> _{TEM} - <i>bla</i> _{CTX-M1-like} - <i>tetA</i>	1/56, 1.8%
<i>bla</i> _{TEM} - <i>aac</i> (3)- <i>II</i> - <i>aac</i> (6')- <i>Ib-cr</i> - <i>qnrB</i>	1/56, 1.8%
<i>bla</i> _{CTX-M1-like} - <i>aac</i> (3)- <i>II</i> - <i>aac</i> (6')- <i>Ib-cr</i> - <i>qnrB</i> - <i>tetA</i> - <i>sulII</i>	1/56, 1.8%
No resistance genes	12/56, 21.4%

Note: ^aPercentage correlated to the total number of commensal isolates (n = 56).

Abbreviations: *E. coli*, *Escherichia coli*; AMR, antimicrobial resistance.

Thus, it may be considered a virulence-like factor in specific ecological niches where the antimicrobial drug-resistant bacteria are particularly able to colonize.³⁹ Unfortunately, the extensive use of antibiotics has changed the natural evolution of bacteria by reducing the susceptible bacterial populations and increasing resistant ones.⁴⁰ Moreover, the genetic background of resistant bacteria allows them to persist in the presence of minimal concentrations of antibiotics.⁴¹

In this study, out of 72 *E. coli* isolates recovered from fecal samples of healthy adult individuals, 56 isolates (77.8%) were identified as gut commensals. These isolates were further analyzed in the current study for antimicrobial susceptibility profiles and mechanisms. Thirty-six (64.3%) of these 56 isolates were MDR that were resistant to at least one antimicrobial agent from three different antimicrobial classes. This finding is concordant with the findings of similar studies, carried out in different countries, that investigated antimicrobial susceptibility of gut *E. coli* in humans, waterfowls and broiler chickens.^{2,4,8,12,42} Many factors contribute to AMR with a complicated inter-relationship that spans across different sectors other than healthcare, such as agriculture and industry. In developing countries including Egypt, the main identified reasons that are leading to AMR in commensal *E. coli* include poverty, overcrowding, socioecological behaviors, highly contaminated waste effluents, food and supply chain safety issues and inadequate surveillance systems.^{43,44} Nevertheless, the principal driver of MDR in these countries is the misuse and over-prescription of antibiotics.⁴⁵ Consequently, commensal *E. coli* typically present in the guts of humans, animals, birds as well as the environmental *E. coli* strains are likely to develop resistance to multiple antimicrobial agents through natural selection when antimicrobial drugs are ingested for the treatment of bacterial infectious diseases.⁴⁴

The present study exhibits a high prevalence of AMR among gut commensal *E. coli* isolates to the most clinically prescribed antimicrobial drugs that are acting with different mechanisms of action. The highest resistance rate of 75% was recorded against each ampicillin and trimethoprim/sulfamethoxazole, followed by ceftriaxone (73.2%), then each cefuroxime, ceftazidime and aztreonam of 71.4% and cefepime of 69.6%. In addition, there was also a high frequency of resistance to amoxicillin-clavulanate (66.1%), fluoroquinolones (62.5%), tetracycline (55.4%) and cefoxitin (64%). However, lower resistance rates to amikacin among isolates were determined (32.1%) and gentamicin (42.9%). These findings were in accordance with the results of many previous studies investigating the AMR profiles of gut commensal *E. coli* but with variable rates from that recorded in the current study. A similar study from Vietnam where gut *E. coli* bacteria, isolated from healthy adults, showed resistance to streptomycin (80.6%), tetracycline (67.0%), ampicillin (65.0%), and trimethoprim/sulfamethoxazole (48.5%).² Moreover, a study that included many countries in South Asia and sub-Saharan Africa regions revealed that commensal *E. coli* isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, streptomycin, tetracycline and norfloxacin with frequencies 65%, 66%, 43%, 56%, 17%, respectively.⁴ Another study from the same geographical area of this study, Egypt, reported high AMR rates among gut *E. coli* isolates from healthy broilers to different antimicrobials including penicillin, erythromycin, trimethoprim/sulfamethoxazole, tetracycline, ceftazidime and amoxicillin/clavulanic acid with frequencies 98.2%, 96.4%, 64.3%, 50%, 41.1%, 26.8%, respectively.⁴⁶ Additionally, another study from Upper Egypt investigated virulence and AMR traits in *E. coli* isolates from broiler chickens showed a high rate of resistance to the majority of the examined antimicrobial agents including 100% resistance rate against each gentamicin, amoxicillin, and quinolones. These isolates showed also considerable resistance rates to oxytetracycline and streptomycin (88%), sulfamethoxazole and trimethoprim (84%) and cefotaxime (76%).⁴⁶ These high levels of AMR in *E. coli* strains from the farm, including resistance to clinically valuable antimicrobials, suggest that *E. coli* might play a significant role as a reservoir for AMR genes and to be a key source for the transfer of AMR traits to other major human pathogens. Consequently, the need for more strict surveillance and improved farming practices (including the regulation of antibiotic usage) is required, which can reduce the carriage of antibiotic-resistant bacteria in foods and thereby minimize the likelihood of HGT of mobile antibiotic resistance genes to other bacteria in the human gut.⁴⁶

The resistance to antimicrobials in bacteria can occur by several mechanisms. One of the main mechanisms is modification or degradation of the antimicrobial drug by the production of enzymes such as β -lactamases, especially ESBLs.⁴⁷ The spread of ESBLs in *Enterobacteriaceae* has become an ever-increasing problem. In *E. coli*, as a member of *Enterobacteriaceae* harboring ESBLs genes, multidrug resistance due to ESBLs production is rapidly becoming a threat to humans.⁴⁸ In developing countries, low levels of sanitation provide opportunities for the transfer of AMR genes in *Enterobacteriaceae*, including *bla*_{CTX-M}, between humans, animals and the natural environment.⁴⁹ The most striking finding in the current study is the widespread of ESBLs production among commensal *E. coli* isolates where a total of

71.4% of *E. coli* isolates was considered as potential ESBLs producers which considered a high percentage even though compared to pathogenic *E. coli* isolated from different clinical infections. A study from Egypt that performed molecular characterization of extended-spectrum β -lactamase-producing uropathogenic *E. coli* revealed that 59.7% of isolates was positive ESBLs producers.⁴⁸ Further, a study reported that 50.5% of isolates were classified as ESBLs-producers from both clinical and environmental *E. coli* isolates.⁵⁰ ESBLs can be broadly divided into three groups, TEM, SHV and CTX-M.⁵¹ ESBL CTX-M has emerged as the predominant type in both humans and animals, commensal, and pathogenic organisms, in addition to community and healthcare-associated infections. In the current work *bla*_{TEM} gene was the most predominant β -lactamase gene (66.1%) followed by *bla*_{CTX-M1-like} gene (42.8%) and *bla*_{CMY-like} genes (10.7%). This result was consistent with the antimicrobial susceptibility results of amoxicillin but slightly differ for other β -lactam antibiotics as illustrated in Table 2. The phenotype-genotype variation may be due to targeting specific β -lactams resistance genes or other resistance mechanisms that were not included in this study.^{42,51} The other main bacterial resistance mechanisms to antimicrobials include prevention of the antimicrobial molecule from reaching toxic levels inside the cell by efflux pumps and modification of the antibiotic target site.^{52,53} These resistance mechanisms can arise through mutations in chromosomal genes or by the acquisition of AMR genes from other bacteria via HGT which is likely the main genetic mechanism of the dissemination of the AMR and virulence encoding genes.¹⁹ HGT may occur through DNA transformation, plasmid conjugation or transduction among bacteria in the high bacterial load environments such as soil as well as the gut in humans and animals.^{53–55}

AMR genes can be carried on different mobile genetic elements, of all, plasmids play an important role in transferring the resistance genes among different bacterial species.⁵⁶ In the present study, plasmid DNA extracts revealed that there were detectable plasmids in 78.6% of isolates and showed different patterns among isolates which comprised from one to three bands of different sizes. This result was comparable to other studies where 80.7% and 60% of *E. coli* and other bacterial species isolates harbored different plasmids carrying AMR genes.^{56,57} The demonstration of harboring plasmids with different sizes among isolates that were resistant to multiple antimicrobials indicates that plasmid-mediated MDR is significant among bacterial pathogens, particularly gut bacteria. Various patterns of AMR genes of other different antimicrobial classes were detected in the present study. The detection of aminoglycoside resistance genes *aac(3)-II*, (42.9%) and *aac(6)-Ib-cr* (25%) was the same pattern for phenotypic resistance to gentamicin but differed for amikacin. This result was different from a study in China where *aac(3)-II* and *aac(6')-Ib-cr* in *E. coli* isolates from waterflows were 27.7% and 72.7%, respectively, also Zhao et al detected *aac(6)-Ib-cr* by 7.5% in *E. coli* isolates from rabbit farms, which was less frequent than our study.^{12,58} The *r* variant of *aac(6')-Ib*, ie, (*aac(6)-Ib-cr*), encodes an aminoglycoside acetyltransferase and also confers resistance to ciprofloxacin by *N*-acetylation of its piperazinyl amine.⁵⁹ Our results revealed that 92.8% of all *E. coli* isolates showed phenotypic resistance to both ciprofloxacin and levofloxacin carriers *aac(6)-Ib-cr* gene. Here quinolones resistance genes *qnrA* and *qnrB* were detected in percentages of 55.4% and 30.4%, respectively, while *qnrS* gene was not found at all. This was less than detected by Zhang et al in China where *qnrB* and *qnrS* were detected in percentages 57.2% and 99.4%, respectively.¹² This difference may be due to the less frequent use of fluoroquinolone in Egypt and/or variable consumption of antimicrobials across countries. In our study, the frequency of tetracyclines and sulfonamide resistance genes were *tetA* (48.2%) and *tetB* (25%), *sulII* (60.7%) and *sulIII* (32.1%), respectively. These rates were in contrast to Zhang et al where *tetA*, *tetB* and *sulIII* were detected in higher percentages of 94.2%, 44.4% and 91.6%, respectively. On the other hand, Zhao et al reported the presence of all sulfonamides resistance genes in the *E. coli* isolates without the coexistence of any tetracyclines resistance genes, which may be attributed to different antibiotics used across different countries and subsequent development of resistance.^{12,58} Additionally, a study from Egypt reported the presence of *tetA* (55%) and *tetB* (40%) in a similar range to our study.⁴² The observed variation could be attributed to the difference in species, or due to different patterns of antibiotic usage across different countries.

In the current work, the presence of multiple resistance genes were detected in 44 (78.6%) isolates, of these isolates, 21.4% and 14.3% carried resistance genes for most common antimicrobial classes; *bla*_{TEM}-*bla*_{CTX-M1-like}-*qnrA-qnrB-tetA-sulII* and *bla*_{TEM}-*aac(3)-II-aac(6')-Ib-cr-qnrA-tetA-tetB-sulII*, respectively. Similarly, Amer et al from Egypt reported multi-resistance genes in 85% of isolates, and 60% of isolates showed the carriage of 4 to 7 resistance genes.⁴² Additionally, a study from Saudi Arabia reported the presence of multi-resistance genes in 99% of *E. coli* isolated plasmids where 15.8% harbored plasmid resistance determinants *bla*_{CTX-M2}, *bla*_{CTX-M9}, *bla*_{CTX-M8}/

²⁵, *rmtB*, *qnrA*, *qnrB* and *qnrS*.⁵⁷ *E. coli* had been reported as a contributor to the dissemination of antibiotic resistance genes in the natural environment.⁶⁰ A key contributor to the dissemination of these clones is the frequent coexistence of *bla*_{CTX-M} with genes conferring resistance to other antimicrobial classes like fluoroquinolones and aminoglycosides, a situation that might lead to high rates of co-selection.⁶¹ The high prevalence of resistance genes has been observed in various reports of animal and human studies.^{2,8,18,62} The presence of numerous antibiotic resistance characteristics on the plasmid is associated with an increase in the presence of pathogenicity features.⁶³ Unfortunately, antibiotic exposure by commensal bacteria certainly increases the risk of the formation of resistant strains that can pass the resistance genes to virulent strains.⁵⁷

Conclusion

The development of MDR bacteria is an emerging problem worldwide, especially in developing countries. The results of the current study revealed a high risk of fecal carriage of antimicrobial-resistant *E. coli* bacteria from healthy adults. *E. coli* isolates showed high resistance rates against diverse antimicrobial agents. Antimicrobial susceptibility profiles revealed that 64.3% of isolates were MDR. In addition, *E. coli* isolates in this study showed high rates of harboring plasmid-carried AMR genes encoding resistance to different antimicrobials. One reason could be due to the over usage or improper use of antimicrobial drugs in Egypt where antimicrobials can be purchased over the counter without a prescription and there is non-compliance with drug law regulations.⁶⁴ Alternatively, it could be due to improper use of antimicrobials in veterinary medicine and for food animals which can be considered a potential reservoir of antimicrobial-resistant bacteria that is transmitted to humans.^{42,46,65} Therefore, it is important to regularly determine the antimicrobial resistance patterns and virulence of microbes to reduce the risk of complications and/or avoid treatment failure in infections caused by MDR *E. coli* strains and the necessity of strict regulation of antibiotics use in Egypt. In addition, based on the results of this study, further investigation of antimicrobial profiles of other gut commensal bacteria is warranted.

Data Sharing Statement

Data and material are available from the corresponding author upon request.

Ethics Approval and Informed Consent

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Heliopolis University (Ethics Approval Code: HU.REC.H.5-2021). Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Disclosure

The authors declare no conflicts of interest for this work.

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