

# Multidrug Drug Resistance of *Escherichia coli* and *Klebsiella* Isolated from Iraqi Patients and Microbiota

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**How to cite this paper:** Hamza, O.A. and Omran, R. (2022) Multidrug Drug Resistance of *Escherichia coli* and *Klebsiella* Isolated from Iraqi Patients and Microbiota. *Journal of Biosciences and Medicines*, 10, 240-252. <https://doi.org/10.4236/jbm.2022.1011019>

**Received:** October 9, 2022

**Accepted:** November 22, 2022

**Published:** November 25, 2022

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## Abstract

**Introduction:** *Escherichia coli* and *Klebsiella* are Gram-negative bacilli of Enterobacteriaceae and are components of the colonic microbiota of animals and humans. The virulent strains cause gastroenteritis and urinary tract infections (UTI), and the incidence of the infections increases due to the increase of multidrug-resistant strains. The aim of this study is to determine the antibiotics resistance profile of *E. coli* and *Klebsiella*. **Methodology:** A total of 100 isolates of *E. coli* and *Klebsiella* were isolated from three sources, healthy stools and patient stools with gastroenteritis and urine subjects with UTI, during the period from November 2021 to January 2022. An antimicrobial susceptibility test was conducted with 14 antibiotics using the disc-Kirby-Bauer's diffusion method. **Results:** Both *E. coli* and *Klebsiella* had variable abilities to resist the studied antimicrobial drugs, including 14 antibiotics belonging to nine different classes that have different patterns or mechanisms in stopping the growth or killing of microorganisms. All bacterial isolates revealed highly significant antimicrobial resistance almost for all antibiotics except carbapenems. About 72% of total isolates were multidrug-resistant (MDR), because they appeared resistant to at least three classes of antibiotics. Only two *E. coli* isolates out of 24 isolates (8.3%) were recovered from healthy stool samples and 6.25% of *E. coli* isolates (2 isolates out of 32) which were obtained from urine samples were sensitive to all antibiotics. The highest rates of antibiotic resistance were observed in *E. coli* than in *Klebsiella*. Both species had resistance to Amoxicillin-clavulanate (70.58%), Cefotaxime (58.96%), and Cefotaxime (57.81%). While the lowest frequency was meropenem (4.86%), and all strains were sensitive to imipenem (100%). **Conclusion:** These results partly explain the high prevalence of antibiotic resistance observed in Iraq due to drug misuse. Most of the bacterial strains were multidrug-resistant,

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and they spread more in pathogenic strains than in commensal strains.

## Keywords

*Escherichia coli*, *Klebsiella*, Multidrug-Resistant, Gastroenteritis, Urinary Tract Infections

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## 1. Introduction

It is widely recognized that antimicrobial resistance (AMR) is a major public health concern worldwide. The antibiotic-resistant *Escherichia coli* and *Klebsiella pneumoniae* are among the most dangerous drug-resistant bacteria. Both species are members of the Enterobacterales and are clinically important, because they regularly cause infections in individuals of all ages. Urinary tract infection (UTI), cholangitis, and sepsis are all infections caused by these bacteria. Currently, both species are becoming more resistant to many antibiotics [1].

Most beta-lactam antibiotics, including penicillins, piperacillin, cephalosporins, amoxicillin-clavulanate, and non-beta-lactam aztreonam, are resistant to extended-spectrum beta-lactamases (ESBLs). MDR bacteria are a good example of antibiotic resistance that is resistant to more than three classes of antibiotics [2]. *E. coli* is facultative Gram-negative bacterium with coccobacilli-shaped cells. Because *E. coli* is commonly found in humans and agriculturally important animals such as cattle, sheep, and goats, it is of particular importance to human and animal health. Most strains of *E. coli* are not pathogenic to humans and animals, but some can cause serious diseases such as diarrhea, urinary tract infections, and other diseases [3].

*Klebsiella* is a Gram-negative, encapsulated, a non-motile bacterium found in the environment and has been linked to pneumonia in groups with diabetes or alcohol use disorder. The bacteria generally colonize the gastrointestinal (GI) tract and pharynx in humans. Once in the body, the bacteria can become highly virulent and resistant to antibiotics. The most common cause of hospital-acquired pneumonia nowadays is *K pneumoniae*. The bacterium is responsible for 3% to 8% of all hospital-acquired bacterial infections in the United States, including pneumonia [4]. Plasmids are essential for the proliferation of CTX-M-type  $\beta$ -lactamases (ESBLs). Among the antibiotic medicines most commonly used to treat bacterial infections are beta-lactams. The development of beta-lactamases that can inhibit the activity of beta-lactam antibiotics is one of the most important approaches to antibiotic resistance that has emerged as a result of extensive exposure of bacteria to beta-lactam antibiotics. The primary factor in beta-lactam antibiotic resistance in Gram-negative bacteria is the production of ESBLs [5] [6]. The gut microbiota of humans can include over 1000 distinct antibiotic-resistance genes, and the transfer of these characteristics across intestinal commensals is a continuous occurrence [7].

The development of antibiotic resistance in Iraq as a result of the misuse of

antibiotics not only reduces the therapeutic efficacy of the drug in treating life-threatening infectious diseases but also increases the overall cost of treatment. Therefore, the ongoing examination of the evolution of bacterial resistance to antibiotics is one of the interesting tasks of microbiologists. This study aimed to investigate the multiple resistance of *Escherichia coli* and *Klebsiella* that cause diarrhea in children and urinary tract infections of all ages and the role of microbiota in transmitting multiple resistance of pathogenic bacteria.

## 2. Methodology

### 2.1. Specimen Collection

The study included 320 specimens collected from the stool and urine of individuals (aged between one to 40 years) of both genders who attended the hospitals of the city of Hillah and the Public Health Laboratory in Babylon, during a period that extended from November 2021 to January 2022.

About 230 stool specimens were collected from two groups, 200 specimens from infants and children with gastroenteritis and 40 stool specimens collected from healthy people, in addition to 90 urine specimens of patients with urinary tract infections for the isolation of *E. coli* and *Klebsiella*.

### 2.2. Strains Studied

Non-repetitive strains *Escherichia coli* and *Klebsiella* isolated from diagnostic specimens of patients who had undergone bacteriological examination in the laboratories concerned were included. The contaminated isolates were excluded.

### 2.3. Data Collection, Transport, and Storage of Strains

Samples of healthy people were collected from the Public Health Laboratory in Babylon, while samples of patients were collected from Hila Hospitals. Data concerning the patient (gender, age, department of origin, strain date and type of sampling, bacterial species) were collected from the records in strict observance of confidentiality. The specimens were transported in a closed cooler tube at room temperature to the laboratory.

### 2.4. Bacterial Isolation and Identification

Serial dilutions ( $10^{-3}$ ) were performed for each stool specimen and precipitated urine by centrifugation, and these dilutions were grown on MacConkey agar plates a selective medium for isolation and identification of enterobacteria, and incubated at 37°C for 24 h. The single pure pink (lactose-fermented) colonies were then selected for subculture on the same medium for further purification. Then pure fermented isolates were grown on Eosin methylene blue (EMB) plates and incubated at 37°C for 24 h to identify the *E. coli* isolates of *Klebsiella*.

Microscopic features of both species were examined using Gram-staining. The biochemical characteristics such as lactose and glucose fermentation, acid production, catalase and oxidase tests urease production, indole production, Methyl

red test, Voges-Proskauer test, and citrate utilization were performed according to [8]. To further identification of *E. coli* and *Klebsiella*, a VITEK<sup>®</sup> 2 system was performed.

### 2.5. Antibiotic Susceptibility

On Mueller Hinton agar, the Kirby-Bauer disc diffusion technique was used to test antibiotic susceptibility. The subsequent antibiotics were examined. Amoxicillin-clavulant (30 µg), piperacilline (100 µg), amikacin (30 µg), ceftazidime (30 µg), cephotaxime (30 µg), Impinine (10 µg), Meropenum (10 µg), ciprofloxacin (5 µg), nitrofurantoin (100 µg), gentamacin (10 µg), Azithromycine (15 µg) Tetracycline (10 µg), Levofloxacin (5 µg), and Chloramphenicol (30 µg) [9] [10].

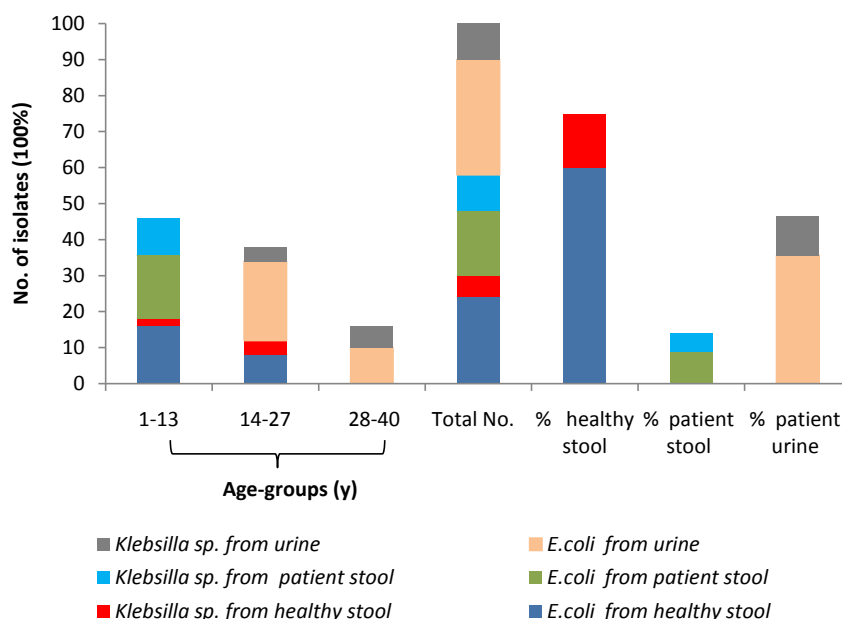
### 2.6. Statistical Analysis

Microsoft Office Excel 2010 software was used to record the data and calculate the frequencies. The qualitative variables were represented in frequency and for the comparison of the variables, we used the Chi-square test. The difference was considered statistically significant for a p-value less than 0.05.

## 3. Results

Out of 200 stool specimens collected from infants and children with gastroenteritis were 18 (9%) positive for *E. coli* and 10 (5%) for *Klebsiella spp.* (Figure 1) and the rest of specimens was negative (86%) which includes other pathogens. The control group includes 24 (60%) *E. coli* and 6 (15%) *Klebsiella spp.* and excluded 25% of specimens. While the percentage of isolation of pathogenic *E. coli* from the patient urine with UTI was 35.56% (32 isolates) and 11.11% (10 isolates) of *Klebsiella spp.*, and the rest specimens (53.33%) containing other pathogens which were excluded.

The results showed that both bacterial species (*E. coli* and *Klebsiella spp.*) have variable abilities to resist the studied antimicrobial drugs, including 14 antibiotics belonging to nine different classes that have different patterns or mechanisms in stopping the growth or killing of microorganisms. All bacterial isolates revealed highly significant antimicrobial resistance almost for all antibiotics except carbapenems, as shown in Table 1. Only two *E. coli* isolates out of 24 isolates (8.3%) were recovered from healthy stool samples and 6.25% of *E. coli* isolates (2 isolates out of 32) which were obtained from urine samples were sensitive to all antibiotics. Although the *E. coli* and *Klebsiella* were isolated from stool samples of healthy people, they showed high resistance to antibiotics and one of them resist eight antimicrobial classes, which indicates the ability of *E. coli* to receive antimicrobial resistance genes from other resistance bacteria by genetic transfer such as transformation, transduction or conjugation in the intestine. Bacteria can acquire antibiotic resistance features from other bacteria within their environment. The 100 *E. coli* and *Klebsiella* bacteria that were isolated from healthy people, gastroenteritis patients, and UTI patients had the



**Figure 1.** Percentage of total Isolates and the isolate number of *E. coli* and *Klebsiella* spp. Isolated from Different Sources according age-groups.

**Table 1.** The proportion of *E. coli* and *Klebsiella* resistant to drugs isolated from healthy and patients with Gastroenteritis.

Antimicrobial categories	Drugs µg/disc	Breakpoints (mm)			% Resistance				
					Healthy stool		Patient stool with Gastroenteritis		Patient urine with UTI
		R≤	I	S≥	<i>E. coli</i> n = 24	<i>Klebsiella</i> n = 6	<i>E. coli</i> n = 18	<i>Klebsiella</i> n = 10	<i>E. coli</i> n = 32
<b>β-lactamase inhibitors/Penicillins</b>	<b>AMC, 30</b>	13	14 - 17	18	62.5	83.3	88.9	80	68.8
	<b>PRL, 100</b>	13	14 - 16	17	50	IR	44.4	IR	12.5
<b>Extended Spectrum 3rd generation cephalosporins</b>	<b>CTX, 30</b>	22	23 - 25	26	58.3	50	55.5	60	50
	<b>CAZ, 30</b>	17	18 - 20	21	58.3	33.3	77.8	80	37.5
<b>Aminoglycosides</b>	<b>AK, 30</b>	14	15 - 16	17	25	33.3	22.2	0	6.3
	<b>CN, 10</b>	12	13 - 14	15	8.3	33.3	11.1	0	18.8
<b>Quinolones/Fluoroquinolones</b>	<b>CIP, 5</b>	21	22 - 25	26	41.7	0	11.1	40	37.5
	<b>LE, 5</b>	16	17 - 20	21	16.7	0	22.2	40	18.8
<b>Carbapenems</b>	<b>MEM, 10</b>	19	20 - 22	23	16.7	0	0	0	12.5
	<b>IPM, 10</b>	19	20 - 22	23	0	0	0	0	0
<b>Macrolides</b>	<b>AZM, 15</b>	12	-	13	41.7	33.3	77.8	40	43.8
<b>Tetracyclines</b>	<b>TE, 30</b>	11	12 - 14	15	33.3	33.3	77.8	60	18.8
<b>Chloramphenicol</b>	<b>C, 30</b>	12	13 - 17	18	16.7	0	44.4	40	6.3
<b>Nitrofurantoin</b>	<b>NIT, 300</b>	14	15 - 16	17	33.3	33.3	11.1	0	25

\* R, resistant; I, intermediate; S, susceptible; IR = inherently resistant; % = percentage. P value < 0.05 calculated for total *E. coli* and *Klebsiella* isolates. AMC: Amoxicillin-clavulanate; PRL: Piperacillin; CTX: Cefotaxime; CAZ: Ceftazidime; AK: Amikacin; CN: Gentamicin; CIP: Ciprofloxacin LE: Levofloxacin; MEM: Meropenem; IPM: Imipenem AZM: Azithromycin; TE: Tetracycline; C: Chloramphenicol; NIT: Nitrofurantoin.

greatest rates of drug resistance. *E. coli* isolates were more resistant than *Klebsiella* with Amoxicillin-clavulanate (70.58%), Cefotaxime (58.96%), and Ceftazidime (57.81%). although the lowest resistance frequency was discovered with meropenem (4.86%), and all isolates were sensitive to imipenem (100%) as shown in the (Table 1).

In Table 1, there are antimicrobial categories, and each category contains one or two types of antibiotic:  $\beta$ -lactamase inhibitors(AMC) the result shows *E.coli* in healthy stool, patient stool, patient urine 62.5, 88.9, and 68.8 rates of resistance respectively, *Klebsiella* in healthy stool and patient stool 83.3, 80 rates resistance respectively. In extended spectrum 3rd generation cephalosporins (CTX, CAZ) the result shows *E.coli* in healthy stool, patient stool, and urine (58.3, 58.3, 55.5, 77.8, 50, 37.5) rates resistance respectively, *Klebsiella* in healthy stool and patient stool 50, 33.5, 60, 80 rates resistance respectively. The results showed that the highest resistance rate in both species was in [AMC, CTX, C AZ], while the results showed that the lowest percentage of resistance in both species in carbapenems (MEM, IPM) the result shows *E. coli* in Healthy stool, Patient stool, Patient urine (16.5, 0, 0, 0 12.5) rates resistance respectively, *Klebsiella* in healthy stool and patient stool (0, 0, 0, 0) rates resistance respectively, with different rates of resistance to the rest of the antibiotics.

The present study found the majority of bacterial strains were multidrug-resistant (MDR) (72%) for at least three classes, including 75.68% MDR-phenotype of *E. coli* and 61.53% MDR-phenotype of *Klebsiella*, and the rest isolates were non-MDR phenotype in addition to two *E. coli* isolates were sensitive to all antibiotics under study as shown in Table 2. Overall, compared to non-MDR strains, MDR bacteria were substantially more likely to exhibit beta-lactam resistance. Amoxicillin-clavulanate ( $p < 0.002$ ), Ceftazidime ( $p < 0.014$ ), Piperacillin ( $p < 0.028$ ) and other antibiotic classes such as macrolides (azithromycin:  $p < 0.001$ ), quinolones/ fluoroquinolones (ciprofloxacin and levofloxacin), tetracyclines and chloramphenicol ( $p < 0.005$ ). Most studied isolates appeared multi-drug-resistant phenotype of *E. coli* and *Klebsiella* isolates, the percentage of MDR-phenotype in *E. coli* and *Klebsiella* which were isolated from urine samples was higher than in patient stools and healthy stool samples (Figure 2). That may be related to the type of isolation sources as indicated by the Chi-Square test  $< 0.005$ .

In Table 2, we performed the statistical analysis using chi-square, and the results showed that there is a significant p-value (0.05) in antimicrobial categories ( $\beta$ -lactamase inhibitors (AMC), Extended Spectrum 3rd generation cephalosporins (CTX, CAZ), Tetracyclines (TE), Macrolides (AZM), Chloramphenicol (C) [0.034, 0.022, 0.022, 0.021, 0.034] respectively. While the rest of the antimicrobial categories has no significant value.

#### 4. Discussion

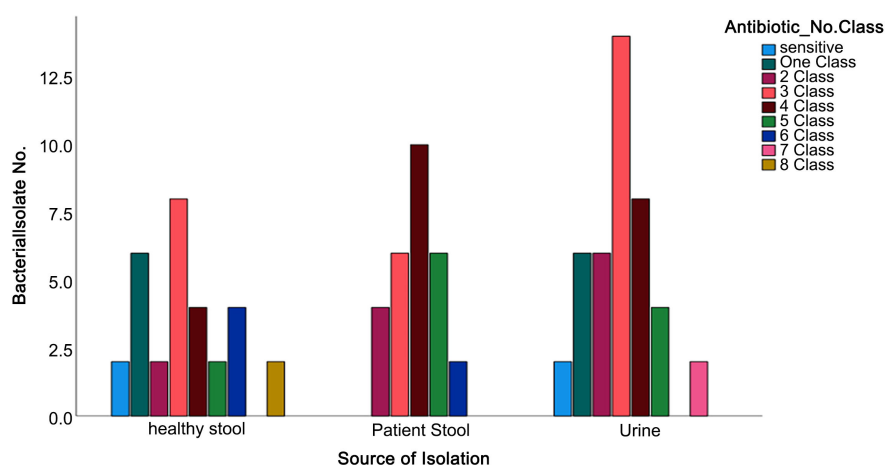
The results of the present study revealed variable patterns of antibiotic resistance. However, the results regarding cephalosporins resistance were not agreed with the results of previous studies that found the resistance of *E. coli* against

ceftazidime and cefotaxime about were variable according to locations, time, and source of isolations. At [9] North Korea discovered the *E. coli* resistance against ceftazidime was 6.8% and 15.5% for cefotaxime. While [11] found the resistance of ceftazidime and cefotaxime were 38.9% and 42.2% respectively. Whereas [12] in Iran reported the resistance of ceftazidime and cefotaxime were 26.1% and 30% respectively. In Egypt, a study found the proportion of cefotaxime and ceftazidime resistance in *E. coli* strains reached 74.4% and 64.3% respectively [13]. And they thought the main reason for the resistance of the two antibiotics may be due to the bacteria having efficient circulating pumps efflux systems that deliver antibiotics outside the bacterial cells. In addition, most pathogenic *E. coli* strains are characterized by their multiple resistance this is due to the transfer of resistance genes among species of the same or closely related genus from the donor to the recipient cell [14].

**Table 2.** Resistance of *E. coli* and *Klebsiella* isolates to antibiotics.

Antibiotics	<i>E. coli</i>			<i>Klebsiella</i>			<i>E. coli &amp; Klebsiella</i>			P* value	P value
	All isolates	MDR Status of Isolates		All isolates	MDR Status of Isolates		All isolates	MDR Status of Isolates			
	n = 74	MDR* n = 56 (75.68%) n (%)	Non MDR n = 18 (24.32%) n (%)	n = 26	MDR n = 16 (61.53%) n (%)	Non-MRD n = 8 (30.77%) n (%)	n = 100	MDR Isolates n = 72 n (%)	Non-MDR n = 26 n(%)		
<b>Pencillins</b>											
<b>AMC</b>	52 (70.27%)	48 (85.71%)	4 (22.22%)	18 (69.23%)	14 (87.50%)	4 (50%)	70 (70%)	62 (86.11%)	8 (30.76%)	<b>0.034</b>	<b>0.002</b>
<b>PRL</b>	34 (45.94%)	34 (60.71%)	0 (0%)	14 (77.78%)	10 (62.50%)	4 (50%)	48 (48%)	44 (61.11%)	4 (15.38%)	0.140	<b>0.028</b>
<b>Fluoroquinones</b>											
<b>LE</b>	12 (16.21%)	10 (17.85%)	2 (11.11%)	8 (30.76%)	8 (50%)	0 (0%)	20 (20%)	18 (25%)	2 (7.69%)	0.119	<b>0.037</b>
<b>CIP</b>	18 (24.32%)	18 (32.14%)	0 (0%)	8 (30.76%)	8 (50%)	0 (0%)	26 (26%)	26 (36.11%)	0 (0%)	<b>0.022</b>	<b>&lt;0.001</b>
<b>Cephalosporis</b>											
<b>CAZ</b>	38 (51.35%)	38 (67.85%)	0 (0%)	18 (69.23%)	14 (87.50%)	4 (50%)	56 (56%)	52 (72.22%)	4 (15.38%)	0.094	<b>0.014</b>
<b>CTX</b>	36 (48.64%)	36 (64.28%)	0 (0%)	20 (76.92%)	14 (87.50%)	6 (75%)	56 (56%)	50 (69.44%)	6 (23.07%)	0.216	0.076
<b>Tetracyclines</b>											
<b>TE</b>	30 (40.54%)	28 (50%)	2 (11.11%)	10 (38.46%)	8 (50%)	2 (25%)	40 (40%)	36 (50%)	4 (15.38%)	<b>0.022</b>	<b>&lt;0.001</b>
<b>Aminoglycosides</b>											
<b>AK</b>	8 (10.81%)	8 (14.28%)	0 (0%)	8 (30.76%)	6 (37.50%)	2 (25%)	16 (16%)	14 (19.44%)	2 (7.69%)	0.257	<b>0.138</b>
<b>CN</b>	12 (16.21%)	10 (17.85%)	2 (11.11%)	8 (30.76%)	8 (50%)	0 (0%)	20 (20%)	18 (25%)	2 (7.69%)	0.119	<b>0.037</b>
<b>Macrolides</b>											
<b>AZM</b>	40 (54.05%)	36 (64.28%)	4 (22.22%)	12 (46.15%)	12 (75%)	0 (0%)	52 (52%)	48 (66.67%)	4 (15.38%)	<b>0.021</b>	<b>&lt;0.001</b>
<b>Nitrofurantoinis</b>											
<b>NIT</b>	16 (21.62%)	12 (21.42%)	4 (22.22%)	2 (7.69%)	2 (12.50%)	0 (0%)	18 (18%)	14 (19.44%)	4 (15.38%)	0.337	0.250
<b>Chloramphenicol</b>											
<b>C</b>	12 (16.21%)	12 (21.42%)	0 (0%)	6 (23.07%)	6 (37.50%)	0 (0%)	18 (18%)	18 (25%)	0 (0%)	<b>0.034</b>	<b>0.002</b>

•Between *Klebsiella* and *E. coli*. A significant p-value (0.05) is in bold. NS stands for “not significant”.



**Figure 2.** Total number of isolates resistant to drug classes according to isolation sources of health and patients with Gastroenteritis and UTI. • Bacterial isolates are resistant to one or more up to 8 classes used in this study including,  $\beta$ -lactamase inhibitors/Penicillins (AMC/PRL), Extended Spectrum 3rd generation cephalosporins (CTX, CAZ), Aminoglycosides (AK, CN), Quinolones/ Fluoroquinolones (CIP, LE), Carbapenems (MEM, IP), Macrolides (AZM), Tetracyclines (TE), Chloramphenicols (C) and Nitrofurantoin (NIT).

The antibiotic resistance of *E. coli* and other bacteria due to bacterial cells had several mechanisms such as the production of  $\beta$ -lactamase enzymes, including cephalosporins and penicillinase, which break down the  $\beta$ -lactam ring of penicillins and cephalosporins, or change the target site of antibiotic by mutations, or decrease outer membrane permeability of bacterial cell, or by efflux pump systems or RND family efflux systems (AcrAB-ToIC, MdfA, YhiV), in addition to inhibiting DNA synthesis for tetracyclines, quinolones group, and aminoglycosides, nitrofurantoin resistances [15] [16] [17].

The development of antibacterial resistance in *E. coli* and other bacteria is multifactorial but has accorded with the incorporation of these agents into the therapeutic resource of human and veterinary medicine. It is largely supposed that antibiotic resistance is the only consequence of human activity and chemotherapy with antibiotics. However, genomic studies of human microbiota and environmental bacteria have revealed large numbers of resistance determinants or genes within their genomes that were not obtained from horizontal transmission and preceded the clinical application of antibiotics in therapy [18]. This type of antibiotic resistance is known as intrinsic resistance and provides selective benefit to the producing strains by inhibiting or eliminating other bacteria that compete for resources. Intrinsic resistance differs from the newly developed exogenous antibiotic resistance in that previously there is no contribution to human activities and the latter is mainly driven by antibiotic selection pressure [19]. Acquired or exogenous and ever-increasing resistance of *E. coli* to antibiotics is a major public health problem worldwide. In 2018, more than half of *E. coli* isolates reported to the European Center for Disease Prevention and Control were resistant to at least one group of antimicrobials under surveillance, and



co-resistance to many antimicrobial groups was frequent (European Centre for Disease Prevention and Control, 2018); In the United States in 2017, the national prevalence of extended-release lactamases (ESBL)-producing strains of *E. coli* isolated from urinary tract infections (UTI) was 15.7%. In developing countries, the situation is getting worse, as reported by national surveillance data from Mexico, China, and Turkey, where it has been proven that resistant strains of *E. coli* spread more than 40% of the widely used cephalosporins and quinolones drugs all over the world for bacterial treatment infection, which agrees with the results of the present study. The present study revealed that the clinical isolates of *E. coli* and *Klebsiella* showed antibiotic resistance more than 57% for cephalosporins and 25% for quinolones. Also, resistance was high in the bacteria colonizing the intestines in healthy subjects as shown in **Table 2**.

Several human activities have been identified as major drivers of the current antibiotic resistance crisis, but the overuse of antibiotics has been shown to influence the development of resistance [20]. The overuse of antibiotics is multifactorial and involves several aspects, including health, livestock, and pharmaceutical industries. Examples of these actions include unsuitable antibiotic prescribing by health care providers, extensive use of antibiotics in livestock and fish farming, patients not following antibiotic regimens, poor hygiene, bacterial mutations, and lack of new antibiotics developed [21]. In addition, global evidence indicates that elements in people's environment such as poor waste, non-potable drinking water, housing overcrowding, and lack of hygiene, facilitate the development and transmission of resistant bacteria [22].

Unfortunately, overuse of these medications and inappropriate prescriptions are major contributors to this problem. In any antibiotic treatment against bacterial infection, the sensitive bacteria will be killed; if pathogenic microorganisms are correctly targeted. However, along with infection with bacteria, the microbiota members of the individual, sensitive to the antibiotics used, will also be eliminated. In the case of resistant microorganisms, either belonging to the normal microorganisms or the target pathogenic microorganisms, these survivors will proliferate and become the dominant strain within the respective anatomical site. Overprescribing medicines is one of the most important factors contributing to the current antibacterial resistance crisis in the rapid evolution of bacteria under selective antibiotic pressure, as the continuous interaction between any given antibiotic and bacteria is an important aspect of increasing multidrug-resistant strains [23]. Inappropriate treatment of acute respiratory infections with ciprofloxacin has led to the development of high rates of *E. coli* resistance worldwide [24]. Also, in Iraq the same circumstances, ciprofloxacin is prescribed to treat several infections such as urinary tract infections and acute respiratory infections that lead to a high rate of resistance against this agent in many pathogenic bacteria such as *E. coli* and *Klebsiella* (**Table 2**). In addition to the contribution of antibiotic abuse to resistance selection [25], found epidemiological evidence that antibiotic resistance and virulence phenotypes of diarrheagenic *E. coli* may be partially related. They found that people with diarrhea were using

antibiotics more frequently before their symptoms appeared, an association that could be interpreted as antibiotics may disrupt the intestinal flora, allowing for the overgrowth of resistant pathogens. [26] found that worldwide antibiotic consumption increased significantly by about 39%, between 2000 and 2015. They also found that the average rate of antibiotic consumption was mainly by low and middle-income earners in developing countries, which leads to the highest rate of antibiotic resistance and prevalence of multidrug inflammatory strains. To make matters worse, the consumption of antibiotics of last resort such as carbapenems and colitis is also on the rise, which is consistent with the emergence of strains of *E. coli* that are resistant to these agents. [27] reported that resistance of *E. coli* to carbapenems is rare, with its prevalence depending on the region of the world under study but does not exceed 3% and it was believed that in the future, increased resistance to carbapenems. It can be observed in *E. coli*, since the enzymes responsible for its degradation, and thus its inactivation, carbapenems, are primarily encoded on conjugative plasmids, and are highly transmissible [28]. This is consistent with the results of the current study, as it was found that the resistance against meropenem had increased to more than 4.5% in *E. coli* isolated from Iraqi people with UTI and healthy stool, as shown in **Table 2**. Antibiotics are also used in livestock to treat clinical diseases and prevent and control common diseases and promote animal growth [29].

Since many antibiotic resistance genes are associated with elements such as plasmids or transposons, and while the transfer of these elements may also occur through transformation or transduction, conjugation is often considered the mechanism most likely responsible for the transmission of these traits [30]. The above-mentioned ESBL and carbapenems genes are prime examples of resistance genes with significant impacts on human health that have spread among bacteria via plasmid conjugation. A study in China [31] showed that transmission by conjugation of ESBL genes in *E. coli* occurs even in the food chain, a situation that partially explains the high fecal prevalence of ESBL-producing *E. coli* around the world.

However, due to the current globalization, resistant strains can easily be transmitted from one country to another. In a large cohort study of Dutch travelers to areas of the world with a high prevalence of ESBL-producing bacteria, 34.3% of people who were ESBL-negative before travel acquired these clones during their time abroad, and the largest number of acquisitions was among those who traveled to South Asia and remained colonized for 12 months after his return [32]. In addition, the same study showed that the estimated probability of transmission within families was 12%. Similar results were reported in a study in Spain, where up to 66% of isolates from patients with ESBL-producing *E. coli* infection were indistinguishable from those isolates from stool samples of their family members [33]. These results indicate that the acquisition of *E. coli*-resistant strains during travel is high and that transmission between family members can maintain these strains in the community for extended periods.

## 5. Conclusion

These results partly explain the high prevalence of antibiotic resistance observed in Iraq due to drug misuse. Most of the bacterial strains were multidrug-resistant, and they spread more in pathogenic strains than in commensal strains.

## Acknowledgements

We express our gratitude to the staff of all the Laboratories concerned for their support and cooperation during this study.

## Availability of Data

All data supporting these findings can be found in the Biotechnology and Genetic Engineering Laboratory at the University of Babylon.

## Authors' Contributions

Ola Adnan as an M.Sc. student perform the methodology of the paper and the supervisor Dr. Rabab Omran planned this research.

## Ethical Considerations

The approvals were obtained from all the participants and also agreed to study the scientific and moral hospitals of Hillah city and the Public Health Laboratory in Babylon. The following information is recorded (patient name, age, gender, date of infection, and chronic disease).

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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