

Screening Study for Some Strains of *E. Coli* Collected from Five regions in Kurdistan-Iraq for its Sensitivity, Resistance and MDR against Thirteen Antibiotics

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Abstract

Background: New strains of bacteria, which are sensitive and resistant to antibiotics, has been selected due to the overuse of antibiotics. The profiles of *Escherichia coli* (*E. coli*), which is resistant to antibiotics, are taken from different samples of human fecal and urine.

Aims: This article investigates the occurrence and observing the sources of *E. coli*, which is sensitive and resistant to antibiotics. The study samples are collected from human urine and fecal by limited inhibition zone.

Results: The study revealed a 92% sensitivity to Chloramphenicol and Gentamycin, 77% to Clarithromycin and Neomycin and 54% to Doxycycline. While the lowest sensitivity level was documented for Erythromycin and Novobiocin represented by 6.8%. Furthermore, the lowest level of sensitivity was reported for Erythromycin, and represented by 38% in comparison to control. Moreover, the sensitivity to penicillin was low. Besides, this study revealed that the isolated samples of *E. coli* were highly resistant to the multiple antibiotic resistance of the three antibiotics, namely Ampicillin, Lincomycin and Rifampin during the same time.

Conclusion: All of the antibiotics used in this study had a clear impact against *E. coli* bacteria as antimicrobial drugs to inhibit its growth and infection.

Keywords: *E. coli*, Antibiotics resistance, Antibiotics sensitivity.

Introduction

E. coli is gram-negative bacilli, facultative anaerobic bacteria that belong to the *Enterobacteriaceae* family¹, and propelled by long flagella. It is found in the intestinal tracts of human and animal due to faecal contamination or food contamination during the slaughter of an animal. It frequently exists in food, water and soil². Furthermore, *E. coli* causes lower urinary tract infection, septicaemia or coleocystis. Resistance to multiple drugs in human pathogenic bacteria has been established because of the random use of commercial antibacterial medicines commonly utilized in treating infectious diseases. In the literature, there are a number of factors impact of developing resistance to antibiotics. These factors include 1) the specific nature of the relationship between bacteria and antibiotics; 2) using an antimicrobial agent; 3) characteristics =of the host; and 4) ecological factors.

Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are used as therapeutic agents³.

Antibiotic resistance

It is well known that the extended-spectrum β lactamases (ESBLs) are produced by multidrug-resistant *Enterobacteriaceae*, commonly *E. coli* (e.g., the enzymes of CTX-M). These enzymes are more active against cefotaxime than other oxyimino-beta-lactam substrates like ceftazidime, ceftriaxone or cefepime. They are defined within the setting of the community as a significant cause of urinary tract infections (UTIs). Recently, there are studies describing ESBL-producing *E. coli* as being a cause of bloodstream infections related to these community-onsets of UTI⁴. It is an organism that occurs widely in sewage because it is a

faecal coliform. It has a significant role in the analysis of water sanitary. Over the past fifty years, two studies conducted by Maaloe and his colleagues have described the growth rate regulation in bacteria for the first time. This has been a significant issue in bacterial physiology⁵. The virulence factor (toxicity) of *E. coli* explains its pathogenicity. There are varied factors that define the pathogenicity of *E. coli*. Based on the nature of these factors, various infections may be caused by these strains, including skin wounds infections⁶.

Pathogenic strains of this organism are distinguished from normal flora by the presence of virulence factors, such as exotoxins. *E. coli*, also known as UPEC, is the most prevalent extra digestive pathogen that causes UTI in children and accounts for about 80-90% of the community-acquired cases. Five pathogenic strains of *E. coli* are frequently isolated from humans and animals suffering from diarrhea. *E. coli* is the most commonly recognized bacterial pathogen in infantile gastroenteritis⁷.

Recently, bacteria species resistant to antibiotics are formal in the environments. The negative effect of bacteria has considerably increased. Resistance to antibiotics in *E. coli* is totally defined in isolates from human, animal and environmental sources. Among the antibiotics, Ampicillin, that belongs to the penicillin family, is used to treat and study these bacteria. The standard Kirby-Bauer disk diffusion method has been employed in this study for determining the profiles of antibacterial sensitivity of the *E. coli* isolates with different antimicrobial agents¹.

Aim and Objectives

This article investigates the occurrence and observing the sources of *E. coli*. The objectives of this work are as follows:

Isolating pure culture of *E. coli* from human fecal samples.

Assessing the antibacterial effectiveness of antibiotics against isolates under study.

Describing resistant strains for the antibiotics used in this study.

Limited multi drug antibiotics (MDA) resistant.

Material and Method

Collection of Stool Specimens

This study included 250 male and female patients. The ages were ranged between 6 months and 5 years. Fifteen samples were selected from 5 years (i.e., positive sample) that included 2 control (not infected). *E. coli* was taken from human fecal. The samples were obtained from hospitals in areas of Soran, Qaladwza, Hawler, Rania and Koya in the Kurdistan region, Iraq. The period of sample collection was during the period from October 2014 to April 2015. The samples were transferred to the laboratories of the research center at the University of Soran. *E. coli* was isolated using the bacteriological and biochemical test of fecal sample collected from patients¹.

The gram-stained slides were obtained from the Central Drug House (P) Ltd. (CDH), which was tested microscopically. Samples were cultivated on Nutrient Broth (Lab M™, Bury, UK) to activate the bacterial isolation of the chemical test reactions (37 °C, 24 h). After that, they were transferred on selective media (Mac Conkey agar, Lab. M. Limited UK) to isolate the rod to the negative gram bacteria and differentiate between fermented and non-fermented isolates of sugar Lactose, Eosin methylene blue (EMB) agar Himedia. The ionic dye contained two blue ones planted by *E. coli*, giving a bright color green. Concerning the final identification, tests of API 20E Biomerieux were used⁸.

Culture media

The preparation of all media utilized in the current study was done in accordance with their manufacturers.

Isolation of bacteria (*E. coli*):

Culture media used were MacConkey agar, EMB agar, Nutrient agar and Blood agar. The standard loop was sterilized and dived in a vertical way after cooling in fecal samples, filed and immediately transported to culture media by duplicate streaking. One of the streaked plates was incubated under aerobic conditions of growth, and the other one was incubated under partial anaerobic conditions of growth with 5% of CO₂. Finally, all streaked plates were placed at 37 °C for 24 hours⁹.

Identification of bacterial isolates

Culture media were prepared for the purpose of purification, conservation, revitalization, quality

appearance identification, cultural recipes and for making a biochemical test of the bacterial isolates. Also, the dyes, solvents and reagents were used to complete biochemical tests as clarified below:

Characteristics of the developed colonies:

The colonies observed on culture media were distinguished according to the following: 1) color; 2) the surface of the colony; 3) strength; 4) transparency; 5) smell; 6) lactose fermentation on Macconkey agar; and 7) the appearance of metallic sheen on EMB agar for *E. coli*⁹.

Microscopic examination:

Smears of each type of appeared colonies were prepared. Gram stain was done to examine the microbial cells gram positivity and negativity as well as cells arrangement⁹.

Biochemical tests:

In the literature, Biochemical tests were done to distinguish *E. coli* and *Staphylococcus epidermidis* from other bacterial isolates. They included Catalase, Oxidase, Coagulase Voges Proskauer, Methyl red, Motility test, Urease, Indol, H₂S Production, Citrate utilization, Sugar fermentation Haemolysis⁹.

Preparation of McFarland turbidity standard No. 0.5

In this study, 50 µl of a 1.175% (wt/vol) barium chloride dihydrate (BaCl₂•2H₂O) solution was added to 9.95 ml of 1% (vol/vol) sulfuric acid to prepare the 0.5 McFarland turbidity standard. Next, the McFarland standard tube was sealed with Parafilm for preventing evaporation. Then, it was stored in a dark place at room temperature. After that, the density accuracy of the prepared McFarland standard was examined through utilizing a spectrophotometer with a 1 -cm light path for the 0.5 McFarland standard, the absorbance at a wavelength of 625 nm and water as a blank standard was 0.08 to 0.13. The turbidity was vigorously agitated by the 0.5 McFarland standard on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland Standard was analogous to a microbial suspension of 1.5 X 10⁸ colony-forming units (CFU)/ml¹⁰.

Antibiotics Tests

Antibiotic discs

High potency discs of the following 13 antibiotics were used against all *E. coli*: Streptomycin (S), Novobiocin (NV), Neomycin (N), Lincomycin (L), Norfloxacin (NOR), Chloramphenicol (C), Doxycycline (DO), Rifampin (RA), Ampicillin (Am), Erythromycin (E), Tetracycline (TE), Gentamycin (CN), Clarithromycin (CLR).

Antibiotic resistance screening test

The resistance of test strains to different antibiotics was determined using the disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) standards. The test strains were first enriched in nutrient broth for 24 h at 37 °C by picking off technique. By using sterile swab sticks, plates were seeded with 1 ml of a suspension of the test strains containing approximately 10⁶ cells. Antibiotic discs were dispensed on the plates seeded with organisms. The plates were incubated at 37 °C for 24 h, and antibiotic resistance was interpreted by inhibition¹¹.

Results and Discussion:

Outcomes of isolation and identification of tested bacteria

There is a significant reason for mortality and morbidity throughout the world, represented by bacterial infectious diseases. Consequently, there is an increasing interest in developing new antibacterial agents for treating bacterial infections.

Table 1: Antibiotic disk.

Antibiotics	Con. Mg	Antibiotics	Con µg.
Ampicillin	10 µg	Rifampin	5µg
Streptomycin	10 µg	Gentamicin	10 µg
Novobiocin	30µg	Chloramphenicol	30 µg
Neomycin30	30 UI	Erythromycin	15µg
Lincomycin	15µg	Tetracycline	30µg
Norfloxacin	10µg	Clarithromycin	15µg
Doxycycline	30µg		

Note: Con.: Concentration

Table 2: Antibiogram of *E. coli* isolates from fecal of different provinces.

Average inhibition zone of diameter size (mm) of <i>E. coli</i> isolates																
Antibiotics	C1	C2	Ave	S 1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
S	1.8	2.8	2.3	1.7	1.7	1.7	1.6	2.4	2.7	1.9	1.5	1.9	1.9	1.7	1.5	1.6
NV	R	0.8	0.4	0.9	0.9	0.9	0.9	1.3	0.5	0.8	2.2	0.9	0.7	1.8	1.7	1.7
N	1.7	1.9	1.8	2	R	R	R	1.6	1.9	1.5	1.7	1.9	1.5	1.6	1.5	1.7
L	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
NOR	2.7	2.8	2.7	3.2	3.2	3.2	2.5	2.7	3	3	2.1	3	2.7	1.9	2.5	2.4
C	2.5	1.6	2	2.5	3	1.6	2	3.2	2	1.2	1.6	2.5	2.1	0.7	1.9	1.5
DO	1.3	R	0.6	3	2.5	R	2	R	R	R	2.5	R	R	1.7	1.9	1.9
RA	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Am	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E	R	R	R	R	R	R	R	R	R	1.2	2.5	R	R	2.5	3	2.1
TE	1.2	R	0.6	R	R	R	R	R	R	R	1.4	1.7	R	R	1.3	1.4
CN	2.3	1.8	2	2.5	2.6	R	1.5	2.6	2.5	1.2	2.5	1.7	0.9	2.5	2.6	2.5
CLR	1.5	1.5	1.5	R	2	R	2	1.2	2	1.8	2.5	2.3	R	2.5	4.3	4.3

**C: Control, **S: Sample, **R: Resistant, Ave: Average

Table 3: The percentage of *E. coli* sensitive to antibiotics isolated from different samples of fecal.

Antibiotics														
Number	C	S	L	NOR	N	NV	TE	CN	CLR	AM	RA	DO	E	
		12	13	0	13	10	5	4	12	10	0	0	7	5
Percentage	92	10	0	10	77	38	31	92	77	0	0	54	38	

Table 4: The percentage of *E. coli* resistant to antibiotics isolated from different samples of fecal.

Antibiotics														
Number	C	S	L	NOR	N	NV	TE	CN	CLR	AM	RA	DO	E	
		0	0	13	0	3	0	9	1	3	13	13	6	8
Percentage	0	0	0	0	23	0	70	8	23	100	100	46	62	

Table 5: The percentage of intermediate resistance of *E. coli* isolated from different samples of fecal.

Antibiotics													
Number	C	S	L	NOR	N	NV	TE	CN	CLR	AM	RA	DO	E
	1	0	0	0	0	8	0	1	0	0	0	0	0
Percentage	8	0	0	0	0	62	0	8	0	0	0	0	0

Table 6: Multiple antibiotics resistance of *E. coli* isolated from different samples of fecal.

Source of isolates	Number of isolates	Number (%) of isolates that showed resistance to multiple antibiotics 3 antibiotics L, RA , Am
Children	13	100%

Prevalence of *E. coli* that is sensitive and resistant to antibiotics isolated from faecal samples:

The results showed that all of the *E. coli* isolates from human fecal are resistant to all medicines at different levels to different antibiotics. As illustrated in Table 2, 53% of the isolates were sensitive to antibiotics, 41% resistant, and 6% intermediate resistant. Some isolates under study showed sensitivity to Erythromycin compared with the control, which gave a negative result. There was a possibility that these isolates were Rfactor. According to Table 3, 92% of Chloramphenicol and Gentamycin, 77% of Neomycin and clarithromycin, 54% of Doxycyclin, 38% Novobiocin and erythromycin, 10% of Streptomycen and Norfloxacin.

Nevertheless, equal amounts (100%) of isolates were found to be resistant to Ampicillin, Lincomycen and Rifampin, 70% of Tetracycline, 62% of erythromycin, 46% of Doxycyclin, 23% of clarithromycin and Neomycin (see Table 4). These results were consistent with those of studies of Masuder (2007), and Zinnah et al. (2008) for above 80% and 53% of the *E. coli* isolates from human fecal were sensitive to Gentamycin, Clarithromycin and Neomycin, respectively. Simultaneously, these studies differ from the current research, as only 8 % of isolates showed resistance to Gentamycin. Also, this study is consistent with that of Wolde Tenssay (2002), which revealed that 100% and 79% of isolates showed resistance to amoxicillin and tetracycline, respectively. Nevertheless, results of this

study are similar to those of in terms of the isolates percentage (50%) that are resistant to tetracycline and due to the insignificant percentage of *E. coli* isolates from human fecal and urine that showed resistance to cotrimoxazole, respectively. Likewise, the current paper was consistent with that of ¹² in that a very unimportant number of isolates reported resistance to norfloxacin. Table 5 shows that the isolates are intermediately resistant to antibiotics depending on the inhibition zone.

Bacteria that were resistant to multiple drugs were taken from fecal samples that were collected at Al-Iklem (see Table 6) represented by 13 *E. coli* isolates examined for revealing resistance to antibiotics. All of them (100%) demonstrated multiple resistance for antibiotics ¹³. Finally, similar studies conducted in and nearby Jimma, Ethiopia indicated that nearly all *E. coli* isolates from environmental sources showed resistance to the frequently used antibacterial medicines including amoxicillin, tetracycline and cotrimoxazole.

Conclusion

From the above findings, we conclude the following:

All antibiotics used in this study had a clear impact against *E. coli* bacteria as antimicrobial drugs to inhibit their growth and infection.

The isolated strains of *E. coli* have a highly sensitive effect on the third-generation aminoglycosides, fourth-generation cephalosporins and carbapenem.

The method of disc diffusion is simple, reliable and low-cost for examining the *E. coli* susceptibility to Am, S, N, L, NOR, C, DO, RA, E, TE, CN, CLR.

Limited MDR depending on the resistant zone, the study found multiple resistance to three types of L, RA and Am.

Conflict of Interest: There is no Conflict of Interest..

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Ethical Approve: This study was conducted with approval from the research ethics committee at the Department of Biology, College of Science, the University of Kirkuk as well as Training and Human Development Division, Department of Health, Kirkuk Governorate.

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