Research Article

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Multidrug Resistant Pattern of *Escherichia coli* Isolated from Cattle, Human Diarrheal Cases and Environment Samples in Basrah Province

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Abstract

The present study aims to diagnose the multidrug-resistant Escherichia coli isolated from human and animal diarrheal cases and environmental samples. The results of bacterial culturing methods revealed that out of 250 collected samples, 53 the isolates exhibited characteristic greenish metallic sheen colonies on EMB agar with bright pink lactose fermenting capabilities on MacConkey agar. Additionally, the conventional PCR assay was used to identify the *E. coli* species based on. T had the uidA gene, and the results showed that all isolates (100%) had the uidA gene. The results showed that all isolates (100%) had the uidA gene, and all isolates (100%) had the target gene. The results also revealed that the incidence rate of MDR *Escherichia coli* is isolated among cattle, humans, and the environment, which have similar rates of resistance for the five classes of antibiotics. Furthermore, MDR *E. coli* isolated from humans, animals, and the environment exhibited a high 100% resistance to amoxicillin-clavulanic acid, as well as (66.66, 83.33, and 72.72) resistance to aminoglycosides (gentamicin and streptomycin), 56.66%, 66.66%, 81.81% resistance to tetracycline (tetracycline), and 66.66%, 50%,100% resistance to dihydrofolate (trimethoprim). In contrast, it showed less resistance to quinolones (Nalidixic acid) 20%, 25%, and 18.18% respectively.

Keywords: *Escherichia coli*, diarrhea, MDR.

Introduction

Antibiotics have been the basis of therapy for bacterial infections since their emergence in the early 20th century. Nevertheless, the widespread development of resistance has created significant challenges. Multidrug resistance is characterized by the lack of susceptibility to at least one antimicrobial agent across three

or more drug classes, as determined through in vitro susceptibility testing (1). In Iraq, antibiotic abuse in hospitals and among farm animals is relatively frequent and common, as it is in many other nations. The uncontrolled and irrational use of drugs has increased multidrug-resistant (MDR) strains. Moreover, multidrug-resistant *Escherichia coli* (MDR) strains have been extensively studied in various regions (2-6). A recent study conducted in Basrah, Iraq, revealed that 58 (93.54%.) out of 62 samples of *E. coli,* isolated from environmental sources and cases of diarrhea, exhibited resistance to a minimum of three distinct antibiotics (7). The results showed high resistance to penicillin, erythromycin, and tetracycline. The resistance rates were 62 (100%), 57 (91.93%), and 50 (80.64%) for each drug, respectively. Tests on sewage samples in Kuwait showed high resistance to cephalosporin antibiotics. They also found genes for Extended Spectrum Beta Lactamase (ESBL) and well-known strains like ST131 and ST648, which could pose health risks to the public (8)., free-range hens in the Caatinga biome of Brazil have been found to harbor cephalosporin-resistant *E. coli*, particularly prevalent in Paraíba. These bacteria exhibit genetic diversity among various taxonomic classifications and possess genes that code for the CTX-M or AmpC enzymes (9). Moreover, recently published research reported that 90% and 57.9% of *E. coli* strains isolated from diarrheagenic animal and human sources, respectively, exhibited a high prevalence of multi-drug resistance, particularly to ampicillin, cefoxitin, imipenem, and ciprofloxacin (10). Additionally, research conducted on clinical isolates from patients in Nigeria demonstrated a notable proportion of *E. coli* strains showing complete resistance to tetracycline and ampicillin, along with over 90% resistance to various other antibiotics evaluated, including levofloxacin, cefuroxime, ciprofloxacin, tobramycin, and amoxicillin/clavulanic acid (11). The spread of multidrug-resistant *Escherichia coli* found in clinical and environmental samples has to be continuously monitored despite these investigations. Therefore, the present study was designed to investigate and identify the frequency of multi-drug-resistant Escherichia coli isolating from diarrheal cases and environmental sources in Basrah, southern Iraq.

Materials and Methods

Sample collection:

From early December 2023 to July 2024, two hundred fifty samples were collected from different regions of Basrah province. The samples were distributed as follows:

Cattle sample: One hundred Fecal samples (1 to 2 grams each) were collected from the veterinary medicine hospital in Basrah province.

Human sample: One hundred stool samples (1 to 2 grams) were collected from the Al-Sadr Teaching and Republican Hospital in Basrah province.

Environmental sample:

Fifty environmental samples (1L) from water and (1gm) from soil farms in Basrah province from December 20230to July 2024. All samples are collected under sterile conditions and immediately transparently in

a cooling box to the Microbiology Laboratory at the College of Veterinary Medicine, Basrah University.

Culturing diagnosis step

The collected samples were first ed on MacConkey agar, subculturing on MacConkey agar, and subculture on Eosin methylene blue (EMB) agar. This step helps to isolate and differentiate the enteric bacteria. The agar plates inoculated with suspected bacteria were then placed in an incubator at a suitable temperature (37°C) for 24hr. Subsequently, the bacterial colonies with different morphologies (size, shape, color, texture) are observed on the agar plates culture to primarily select the suspected colonies for the successful analysis step.

Molecular diagnosis step

DNA Extraction Spin Kit from Anatolia company (Turkey) was used to extract the bacterial DNA based on a silica membranning column that facilitates the extraction and purification of bacterial nucleic acids (12). The traditional PCR was subsequently performed to detect the uidA gene, which encodes the β-glucuronidase enzyme, across all *E. coli* species. A specific foreword primer (F: 5-CCAAAAGCCAGACAGAGT-3) and reverse primer (R: 5-GCACAGCACTTCAAAGAG-3) were employed to achieve this goal. A total volume of 25 µl of PCR reaction tubes was prepared and consisted of 12.5 µl of premix hot-start enzyme, 1 µl (10 pmol) of each forward and reverse primer and 4 µl of DNA template. Subsequently, the final volume was accomplished by adding nuclease-free water. A PCR apparatus was used for the PCR amplification steps, starting with a 5-minute pre-PCR heating step at 95°C. Then 35 cycles, each consisting of one minute at 94°C, one minute at 58°C, and one minute at 72°C, then another 72°C for five minutes as a final extension (13). The size of the amplified product was verified utilizing a red-safe DNA solution in a 2% agarose gel electrophoresis (14).

Antimicrobial sensitivity test

Using the conventional Kirby-Bauer disc diffusion method outlined in the Clinical and Laboratory Standards Institute (CLSI) recommendations, all presumed pure colonies of *E. coli* were tested for their susceptibilities against the selected antibiotics (15). As indicated in Table 1, the bacterial isolates were examined for antibiotic resistance to six antibiotics from five distinct classes.

Table 1: Types of antibiotics and their classes

Antibiotic classes	Antibiotic	Symbol	Conc.
Quinolones	Nalidixic acid	NA	30µg
Penicillins	Amoxicillin-clavulanic acid	AMC	30 µg
Aminoglycosides	gentamicin	GN	10 µg
	streptomycin	S	10 µg
Tetracyclines	tetracycline	TE	30µg
Dihydrofolate	trimethoprim	TR	5 µg

Results

Culturing diagnosis results

Both traditional and molecular approaches were used to detect E. coli in collected samples. The culturing results showed that 53(30 from cattle, 12 from humans, and 11 from the environment) exhibited a distinctive greenish metallic sheen appearance on EMB agar, while on MacConkey agar, the suspected colonies were growing to have a rounded, non-mucoid, bright pink (lactose fermenting) texture.

Molecular diagnosis results

The typical PCR technique was used to detect the presence of the uidA gene in the suspected *E. coli* isolates, and the results revealed that 53(100%) of isolates carried the target gene (Figure 1).

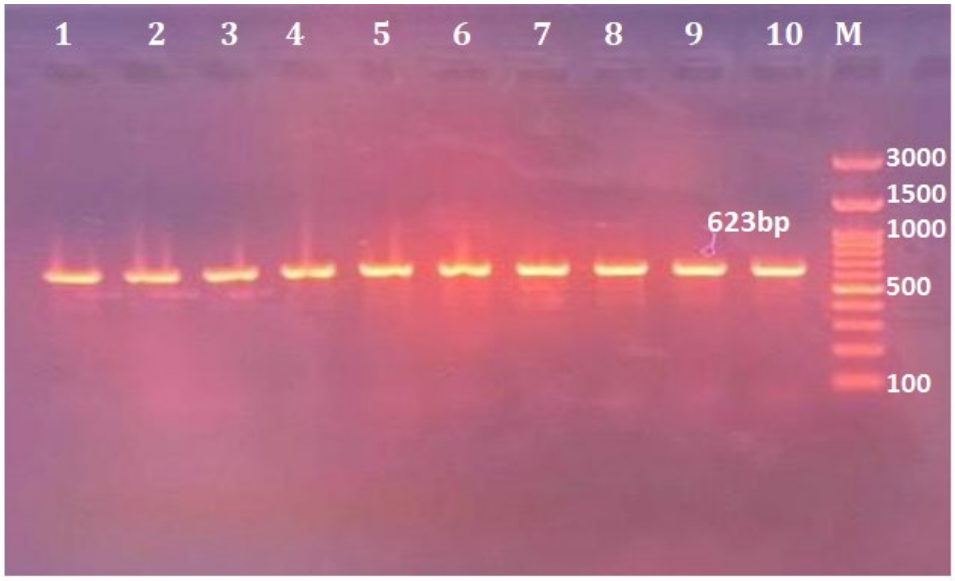

Figure 1: PCR products of the *uidA* gene of *E. coli*. The size of the PCR product is 623bp. M: Marker DNA ladder (100bp-3000bp).

Antimicrobial susceptibility testing results: A total of 53 *E. coli* isolates were examined for antibiotic resistance, comprising 30 from cattle, 12 from humans, and 11 from environmental sources. The assessment used the Kirby-Bauer disk diffusion method against six antibiotics from five distinct classes. Among the 30 isolates from cattle, varying levels of resistance were observed: 100% exhibited resistance to amoxicillin-clavulanic acid, which belongs to the penicillin class; 66.66% and 50% showed resistance to gentamicin and streptomycin, respectively, both of which are aminoglycosides; 56.66% were resistant to trimethoprim, associated with the dihydrofolate class; and 66.66% demonstrated resistance to tetracycline, categorized under tetracyclines (Table 2) and (Figure 2).

Table 2: The multidrug resistance of *E. coli* isolated from cattle sample

Antibiotic classes	Antibiotics*	Resistant		Intermediate		Sensitivities	
Quinolones	NAL	<13	6(20%)	14-18	9(30%)	>19	15(50%)
Penicillins	AMC	<13	30(100%)	14-17	0(0%)	>18	0(0%)
Aminoglycosides	GEN	<14	20(66.66)	15-17	7(23.33)%	>18	3(10%)
	Strep	<11	15(50%)	12-14	8(26.66%)	>15	7(23.33%)
Tetracyclines	TET	<11	17(56.66%)	12-14	12(40%)	>15	1(3.33%)
Dihydrofolate	TMP	<10	20(66.66%)	11-15	10(33.33%)	>16	0(0%)

***Nalidixic acid: NAL; Amoxicillin-clavulanic acid: AMC; Gentamicin: GEN; Streptomycin: Strep; Tetracycline: TET; Trimethoprim: TMP.**

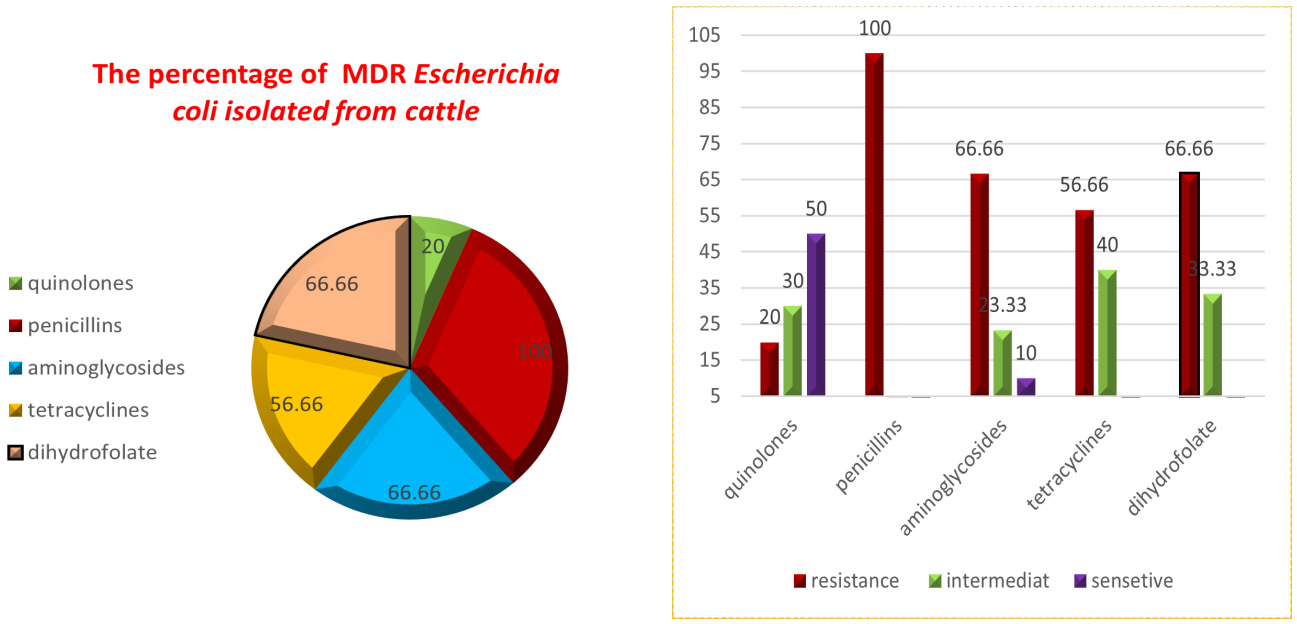

Figure 2: The percentage of MDR *Escherichia coli* isolated from cattle samples

On the other hand, Table (3) and Figure (3) demonstrate the results of MDR *E. coli* in 12 human isolates. The results showed varied levels of resistance to antibiotics, in which 100% of isolates was resistant to amoxicillin-clavulanic acid, which represents the penicillin's class. In comparison, 83.33% and 75% of isolates were resistant to gentamicin, streptomycin related to aminoglycosides class. In

addition, 66.66% of isolates were resistant to tetracycline representing the tetracyclines class and 50% of isolates were shown resistance to trimethoprim related to dihydrofolate class. Moreover, the results of the antibiotic resistance susceptibility test of *E. coli* isolated from 11 environmental samples were showed also different levels of resistance in which 100% of isolated bacteria were resistant to amoxicillin-clavulanic acid and trimethoprim, which related to penicillin's and dihydrofolate classes. In contrast, 72.72% and 63.63% of isolates were resistant to gentamicin, streptomycin is related to aminoglycosides (Table 4) and (Figure 4).

Table 3: The multidrug resistance of *E. coli* isolated from human sample

Antibiotic classes	Antibiotics*	Resistant		Intermediate		Sensitivities	
Quinolones	NAL	<13	3(25%)	14-18	4(33.33%)	>19	5(41.66%)
Penicillins	AMC	<13	12(100%)	14-17	0(0%)	>18	0(0%)
Aminoglycosides	GEN	<14	10(83.33%)	15-17	2(16.66%)	>18	0(0%)
	Strep	<11	9(75%)	12-14	3(25%)	>15	0(0%)
Tetracyclines	TET	<11	8(66.66%)	12-14	4(33.33%)	>15	0(0%)
Dihydrofolate	TMP	<10	6(50%)	11-15	6(50%)	>16	0(0%)

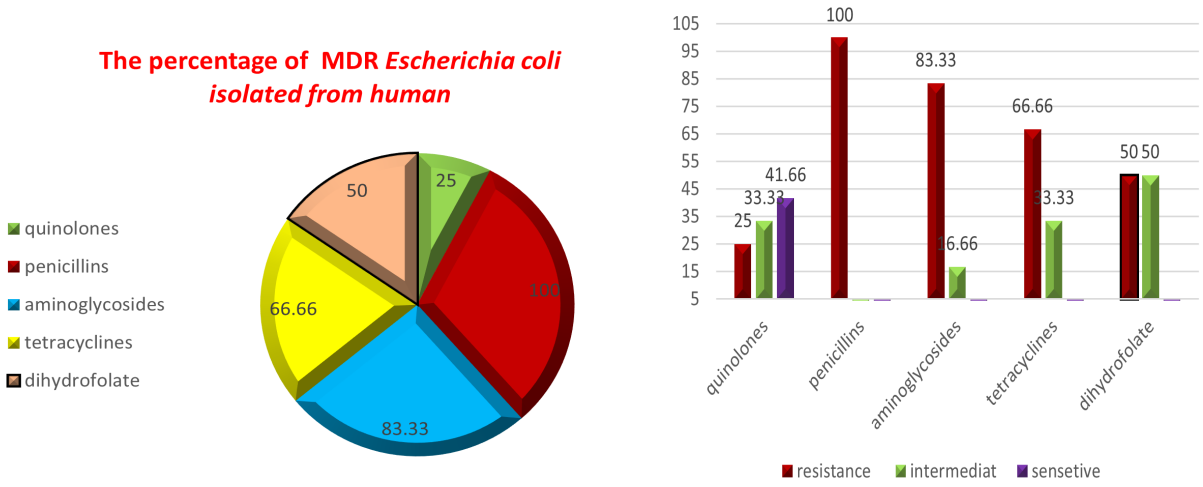

Figure 3: The percentage of MDR *Escherichia coli* isolated from human samples

Table 4: The multidrug resistance pattern of *E. coli* isolated from an environmental sample

Antibiotic classes	Antibiotics*	Resistant		Intermediate		Sensitivities	
Quinolones	NAL	<13	2(18.18%)	14-18	0(0%)	>19	9(81.81%)
Penicillins	AMC	<13	11(100%)	14-17	0(0%)	>18	0(0%)
Aminoglycosides	GEN	<14	8(72.72%)	15-17	0(0%)	>18	4(36.36%)
	Strep	<11	7(63.63%)	12-14	4(36.36%)	>15	0(0%)
Tetracyclines	TET	<11	9(81.81%)	12-14	0(0%)	>15	2(18.18%)
Dihydrofolate	TMP	<10	11(100%)	11-15	0(0%)	>16	0(0%)

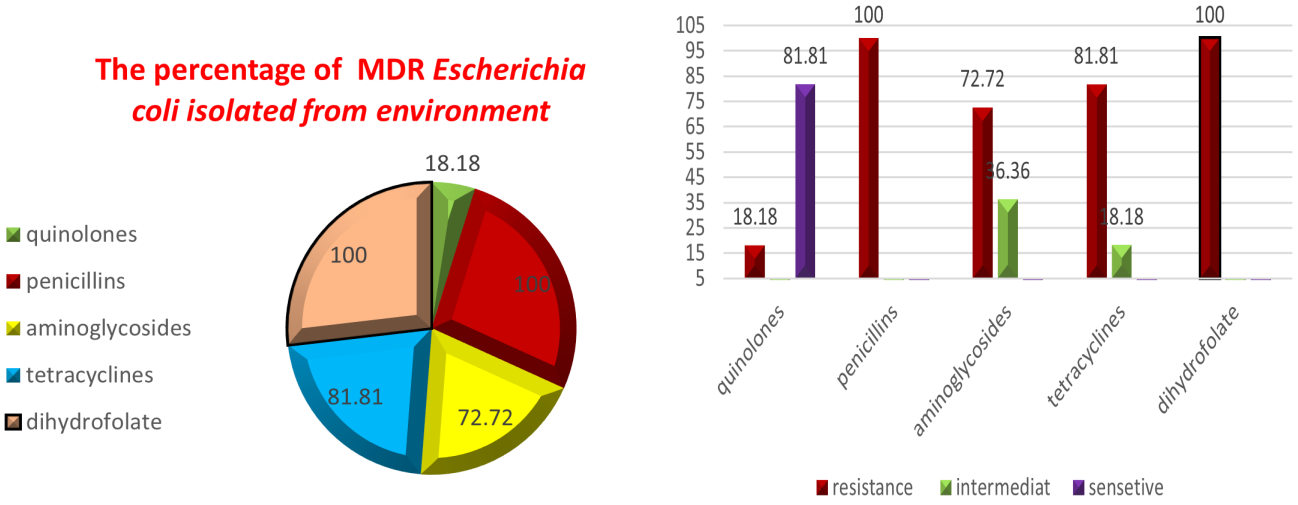

Figure 4: The percentage of MDR *Escherichia coli* isolated from environmental samples

Furthermore, Table (5) and Figures (5) and (6) revealed the incidence rate of *Escherichia coli* isolated among cattle, humans, and the environment, which seem to have similar rates of resistance for the five classes of antibiotics and antibiotic resistance profile**.** Table (6) presented the antimicrobial resistance pattern of *E. coli* that recovered from cattle, humans, and environment. These isolates were distributed into 31 patterns. he most commonly observed resistance patterns were NA - AMC - GN - S - TE - TR. The highest multi-resistance was observed, with 20.75% of the isolates (11 in total) exhibiting resistance to six different antimicrobials (Table 6).

Discussion

Antimicrobial resistance within the Enterobacteriaceae family, especially E.

coli, poses a global health risk (16). Furthermore, the risk of E. coli is increasing, as this bacterium can be transmitted from animals to humans through direct contact. This is particularly evident among workers in large animal slaughterhouses, those with occupational exposure on farms, and through ingesting contaminated food. Moreover, *E. coli* can also be transmitted to humans indirectly through environmental routes (17)

Table 5: The percentage of resistance *Escherichia coli* isolates isolated from cattle, humans, and the environment.

Antibiotic class	Antibiotic	Cattle	Human	Environment
Quinolones	Nalidixic acid	20%	25%	18.18%
Penicillins	Amoxicillin-clavulanic acid	100%	100%	100%
Aminoglycosides	gentamicin	66.66	83.33%	72.72%
	streptomycin	50%	75%	63.63%
Tetracyclines	tetracycline	56.66%	66.66%	81.81%
Dihydrofolate	trimethoprim	66.66%	50%	100%

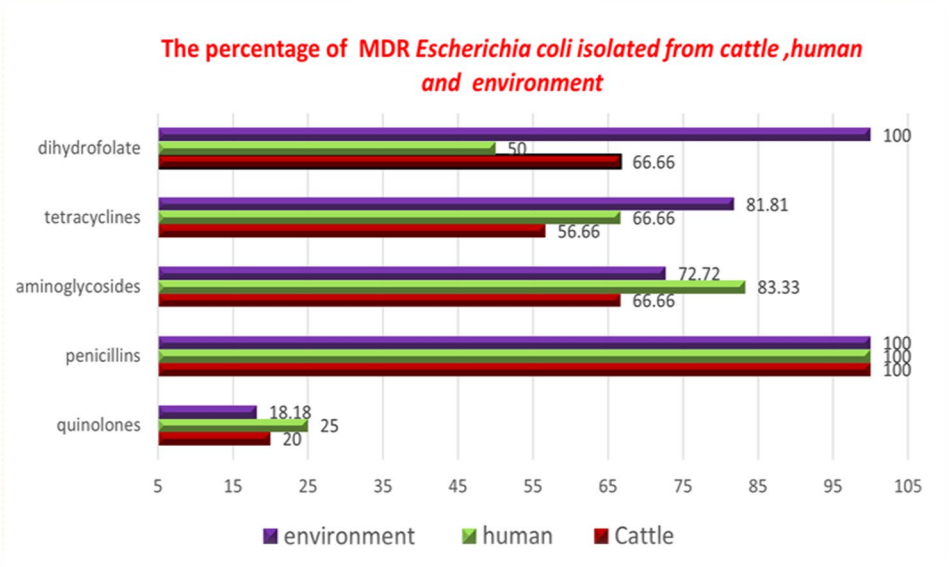

Figure 5: The percentage of MDR *Escherichia coli* isolated from cattle, humans and the environment.

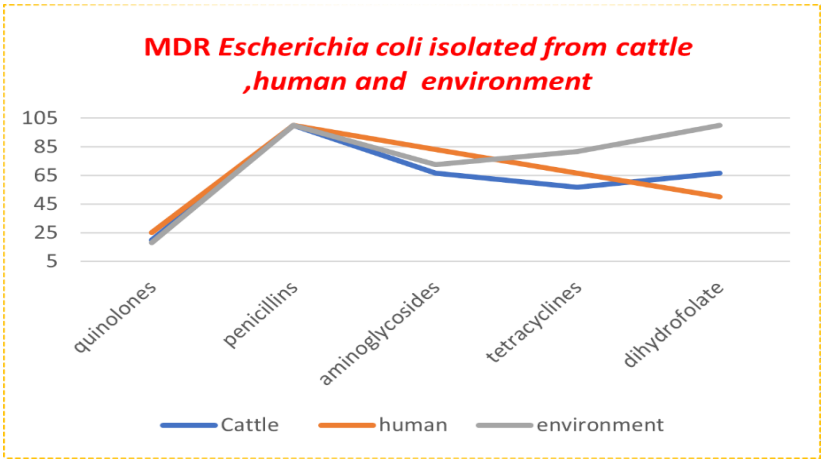

Figure 6: The correlation of MDR *Escherichia coli* isolated from cattle, humans and the environment with 5 antimicrobial agents.

In the current investigation, the findings from bacterial isolation indicated that among a total of 100 fecal samples collected from cattle, 100 stool samples from humans, and 50 environmental samples, 53 isolates demonstrated unique greenish metallic sheen colonies on EMB agar, along with rounded, non-mucoid, bright pink colonies exhibiting lactose fermentation on MacConkey agar. Additionally, the suspected bacterial isolates were identified through a conventional PCR assay targeting the uidA gene, which is universally present in all *E. coli* species. All isolates (100%) exhibited positive results, which agrees with the results of (18). However, our research indicates a higher percentage of *E. coli* isolated from animals compared to previous studies, which reported isolation rates of 4.7% and 5% (19). These findings could be attributed to the difference in findings between studies due to the use of dairy farm management measures, which prevent harmful bacteria from spreading throughout dairy cows. Cleaning the farm floor, all tools used, and providing safe water for dairy farms are important to prevent *E. coli* from growing and spreading among cows, which helps reduce the risk of bacterial contamination and infection (20). On the other hand, the results demonstrated that the local *Escherichia coli* isolates recovered from cattle, humans, and the environment had similar rates of resistance to antibiotics for the five classes of antibiotics. This finding was consistent with (10), who discovered that human isolates demonstrated high resistance MDR patterns and that resistance to specific antibiotics might be attributable to exclusively treating humans and animals with that particular antibiotic. Moreover, (7) their study shows a multi-significant frequency of antibiotic resistance in *E. coli* that is isolated from diarrheal cases in both humans and animals. Antibiotic resistance presents a global challenge that poses significant risks tohumans' and animals' health and well-being (21).

Table 6: Antibiogram correlation profile results of 53 *E. coli* isolates isolated from cattle, humans, and the environment distributed into 31 pattern types

Patterns	Profile	Numbers of resistance antimicrobials	Numbers of resistance isolates from 53	Percentage % From 53 isolate
1.	NA - AMC - GN - S - TE - TR	6	11	20.75%
2.	NA - AMC - GN - S - TE	5	2	37.73%
3.	NA - AMC - GN - S - TR	5	2	
4.	NA - AMC - GN - TE - TR	5	3	
5.	NA - AMC - S - TE - TR	5	1	
6.	NA - GN - S - TE - TR	5	4	
7.	AMC - GN - S - TE - TR	5	8	
8.	NA - AMC - GN - S	4	0	5.66%
9.	NA - AMC - GN - TR	4	0	
10.	NA - AMC - TE - TR	4	0	
11.	NA - S - TE - TR	4	0	
12.	GN - S - TE - TR	4	0	
13.	AMC - GN - TE - TR	4	3	
14.	NA - AMC - GN	3	0	5.66%
15.	NA - AMC - TR	3	0	
16.	NA - TE - TR	3	0	
17.	S - TE - TR	3	0	
18.	GN - S - TR	3	0	
19.	AMC - GN - TE	3	3	
20.	NA - AMC	2	0	1.885
21.	NA - TR	2	0	
22.	TE - TR	2	0	
23.	S - TR	2	0	
24.	GN - TE	2	0	
25.	AMC - GN	2	1	
26.	NA	1	0	28.305
27.	TR	1	0	
28.	TE	1	0	
29.	S	1	0	
30.	GN	1	0	
31.	AMC	1	15	

A report by (22) indicates that if this issue remains unaddressed, it is projected that by 2050, antibiotic-resistant bacteria could result in an annual increase in mortality rates, potentially affecting up to 10 million individuals. Pathogenic bacteria exhibiting resistance can emerge in humans, animals, and the environment, posing a threat to public health due to the imprudent use of antibiotics, including both misuse and overuse, across different sectors such as livestock and human environments. In addition to antibiotic resistance in pathogenic bacteria, it has also been identified in commensal bacteria, including *E. coli.* This point of view is also shared by (23), which indicates that one of the

commensal bacteria, *E. coli,* has developed resistance to more than one type of antibiotic. (24) found that *E. coli* can transfer antibiotic-resistant genes to other bacteria, including dangerous bacteria (25). On the other hand, bacteria that are resistant to at least one antimicrobial agent in three or more antimicrobial groups are known as multi-drug resistant (MDR) microorganisms. The current research demonstrated a rise in multiple drug resistance (MDR) across more than one antibiotic within five distinct antimicrobial categories. The findings indicated that the *Escherichia coli* isolates obtained from cattle, humans, and environmental sources exhibited comparable rates of antibiotic resistance across the five classes. Our research aligns with the findings of the study (26), which reported that almost 90% of isolates were multidrug resistant against at least three antibiotics and were identified in diarrheal samples collected from cattle farms in Pakistan. This study also indicated a significant level of antibiotic resistance, predominantly to (100%), ampicillin (100%), sulfamethoxazole-trimethoprim (85%), and tetracycline. Furthermore, the prevalence of multidrug-resistant *E. coli* was 57.3%, characterized by 39 distinct resistance patterns.

CONCLUSION

In conclusion, the emergence of pathotypes and multidrug-resistant *E. coli* infection in livestock is considered a public health issue in Basrah province.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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نمط المقاومة للأدوية المتعددة في الاشريكية القولونية المعزولة من الماشية وحالات الإسهال البشري وعينات البيئة في محافظة البصرة

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الخلاصة

تهدف الدراسة الحالية الى تشخيص بكتريا الاشريكية القولونية المتعددة المقاومة المعزولة من الاشخاص المصابين بالإسهال وحالات الاسهال في الابقار ومن العينات البيئية ، وكشفت نتائج الاستزراع البكتيري أنه من بين 250 عينة تم جمعها ، أظهرت 53 من العزلات مستعمرات لامعة معدنية مخضرة مميزة على وسط EMB , وذات لون وردي زاهي مع قابلية تخمير اللاكتوز على وسط .MacConkey بالإضافة إلى ذلك ، تم استخدام اختبار تفاعل البوليمير المتسلسل التقليدي لتحديد أنواع الإشريكية القولونية اعتمادا على وجود جين uidA ، وأظهرت النتائج أن جميع العزلات (100٪) لديها الجين المستهدف. كما كشفت النتائج أيضا عن معدل حدوث الإشريكية القولونية المتعددة المقاومة و التي تم عزلها من الماشية والانسان والبيئة حيث اظهرت معدلات مقاومة متماثلة لفئات المضادات الحيوية الخمس المستخدمة في التجربة. بالاضافة الى ذلك فقد اظهرت الإشريكية القولونية المتعددة المقاومة المعزولة من الماشية والانسان والبيئة مقاومة عالية بنسبة 100٪ لحمض الاموكسيسيلين الكلافولانيك ، بالإضافة إلى (66.66 ، 83.33 ، 72.72) مقاومة للأمينوغليكوزيدات (الجنتاميسين والستربتومايسين) ، 56.66٪ ، 66.66٪ ، 81.81٪ مقاومة للتتراسيكلين (التتراسيكلين) ، و 66.66٪ ، 50٪ ، 100٪ مقاومة لثنائي هيدروفولات (تريميثوبريم) ، بينما أظهر مقاومة أقل للكينولونات (حمض ناليديكسيك) 20٪ ، 25٪ و 18.18٪ على التوالي.

الكلمات المفتاحية: الاشريكية القولونية , الاسهال, المقاومة المتعددة للمضادات الحيوية.