

Original article

Isolation and Immunological Characterization of Antibiotic-Resistant *Escherichia Coli* from Human and Animal Sources in Libya

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Abstract

Recently, *Escherichia coli* has become a public health concern worldwide. This is because there has been increasing resistance both among animals and among human beings. The main aim of the present study was to isolate antibiotic-resistant *Escherichia coli* from animal and human sources in Libya. In this research, a cross-sectional design has been adopted, where 100 samples have been collected proportionally from both human and animal sources. The animal samples were meat samples, raw milk samples, and animal excrement. Antibiotic susceptibility testing was performed using the disc diffusion test. Also, immunological testing was performed for immunological characterization. Resistance to ampicillin (68.0%), tetracycline (61.0%), and cephalosporins (57.0%) was identified for the isolated bacteria. In particular, it is important to indicate that 63.0% of the isolated bacteria were multidrug-resistant *E. coli*. It should be noted that 58.0% of the isolated organisms are virulent. Similarity in terms of resistance patterns between humans and animals has been observed.

Keywords. *Escherichia Coli*, Antibiotic Resistance, Multidrug Resistance, Immunological Characteristics.

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Introduction

The pathogen causes diseases in humans and animals. In view of the fact that many different diseases can occur due to the presence of *E. coli*, one can say that pathogens of the microorganism are dangerous for people [1]. However, some strains of the microorganism do not affect human well-being and serve as part of the normal gut microbiome. The use of antibiotics has led to a substantial increase in the number of MDR *E. coli* over the last twenty years. From a One Health perspective, it could be argued that the pathogens can develop resistance to antibiotics not only in people but also in animals. As was found in research, the presence of resistant bacteria in animal hosts may help to transfer antibiotic-resistant genes in humans [2], [3].

In Libya, there has been an increase in cases of antibiotic resistance due to improper use of antibiotics, lack of infection control measures, and failure to put in place monitoring mechanisms. Antibiotic resistance has been seen to develop in some of the strains of *E. coli* that resist common types of antibiotics in Libya, according to some scientists. Through the study carried out in Benghazi, it is evident that strains of the bacteria *E. coli* account for the most causative agent of uropathy in the country and have developed resistance to widely used drugs such as amoxicillin, cotrimoxazole, ciprofloxacin, and cephalosporins [4]. Evidence on the transmission of resistant microorganisms away from the hospitals in a clandestine manner may be obtained from the prevalence rate of ESBL-producing *E. coli* strains isolated from children and healthy subjects in Libya [5]. There have also been some cases of the presence of *E. coli* bacteria in the livestock, meat, milk, and other animal products in Libya [6]. Nevertheless, there remain numerous knowledge gaps regarding antibiotic-resistant *E. coli* infections in Libya. In the previous literature, most works have considered the subject individually for human clinical isolates or animal isolates [7]. Also, it should be noted that not many scientists have considered the study of the immunological features of *E. coli* strains, their level of resistance, and the potential threat of infection among various hosts within Libya. In [8], there are limited molecular and immunological studies available on the mechanism by which such resistant strains survive and propagate amongst various hosts in Libya.

Hence, the current study aims at screening and immune characterization of resistant *E. coli* strains isolated from human and animal sources in Libya. The objectives of the study are to determine the prevalence of resistant isolates and their antibiotic resistance pattern. This study also intends to perform immunological and pathogenic characterizations of the isolates to find out the mode of transmission through comparison between human and animal isolates.

The innovative feature of this research is its combination of the "One Health" approach through the simultaneous study of antibiotic-resistant *E. coli* isolates in both humans and animals from the Libyan environment. Previous studies mostly

centered on the study of the prevalence and/or antibiotic resistance patterns of *E. coli* isolates while this study utilizes an integrated approach involving bacterial isolation together with immunology. Conclusions drawn from this study will play an important role in providing scientific information regarding infection control and prevention in Libya.

Human and animal intestines are commonly used to identify Gram-negative bacteria such as *E. coli*. While some pathogenic types may pose no threat to their host organism, others may result in life-threatening diseases like meningitis, sepsis, gastroenteritis, and urinary tract infections [9]. There has been an increase in *E. coli* cases resistant to antibiotics in recent decades due to the misuse of antibiotics by humans and veterinarians. It has been established that *E. coli* exhibits resistivity. There has been an increase in cases of *E. coli* that is resistant to normal drugs like tetracycline, cephalosporin, penicillin, and fluoroquinolone [10].

In numerous scientific studies, it is indicated that animals act as significant sources of resistant *E. coli*. Resistance to antibiotics among bacteria develops as a result of their continuous exposure to antibiotics through their use to encourage growth and protect against infections within the agricultural industry. Such infections may be caused by humans from animals in agriculture, either directly by contact or indirectly by consuming the meat of these animals. The spread of antibiotic resistance is demonstrated through the concept of "One Health," which states that antibiotic resistance extends to people, animals, and the environment [11,12].

Nevertheless, previous studies done in Libya have identified *E. coli* bacteria that are resistant to antibiotics in both humans and animals. This has been confirmed by hospital-based studies where human specimens exhibited resistance to ampicillin, amoxicillin, and cotrimoxazole antibiotics. Likewise, in studies performed using meat, dairy, and livestock samples, the pathogenicity and resistance of *E. coli* were determined. It should be noted that most of the studies carried out in Libya were mostly concerned with the isolation and susceptibility testing of bacteria, but very little was done regarding their immunity and virulence [13]. Moreover, there have been very few comparative studies analyzing strains of resistant *E. coli* that are derived from humans and animals at the same time. Due to the absence of integrated studies, there is little knowledge about the epidemiological linkages and the mode of transfer among hosts. Therefore, further studies should be done to assess the immunological properties and resistance of *E. coli* in both humans and animals from Libya [14].

Methods

Study design and sampling

For the purposes of this experiment, the method that is going to be used to identify and characterize the drug-resistant strains of *E. coli* in humans and animals within Libya is a cross-sectional design involving a laboratory-based approach. Samples will be collected from different parts of Libya during the course of the study period and will include hospitals, health centers, livestock farms, and slaughterhouses. In addition, samples collected from patients who are suspected of being infected with bacteria will include urine samples, fecal samples, and wound swabs.

Sampling for humans and animals will be done using purposive sampling, where the subjects to be sampled will be relevant to the research objectives. Contamination during sample collection will be prevented through the use of sterile swabs and sterile storage containers. The samples will be taken and transported to the microbiology laboratory for analysis within a span of 24 hours. *E. coli* isolation will involve the use of traditional bacteriology approaches such as culturing on MacConkey agar plates and EMB agar, followed by other bacterial identification tests like indole, citrate, methyl red, and Voges-Proskauer. Determination of antibiotic sensitivity will involve the Kirby-Bauer disk diffusion test in accordance with the CLSI standards.

Data collection

The collection of the data shall involve two sources, which are laboratory and demographic data. For laboratory data, the following will be collected: number of *E. coli* isolates obtained, antimicrobial resistance profile of *E. coli*, multi-drug resistance of *E. coli*, and immuno-characterization of *E. coli* isolates. The other data that shall be collected are demographic data such as age, gender, sampling sites, species of animals, and the collection site of samples. Additionally, conducting tests for antibiotic sensitivity of the *E. coli* isolates to various antibiotics such as ampicillin, tetracycline, ciprofloxacin, gentamicin, and cephalosporins will constitute another component of data collection. Serotyping as well as immunoassay for virulence-associated antigens may constitute the immuno-characterization process of the isolates [15].

Data analysis

Version 26 of the Statistical Package for the Social Sciences (SPSS) or an equivalent software tool will be employed to input, analyze, and clean the collected data. To determine the prevalence and distribution of the resistant strains of E. Coli, descriptive statistics such as frequency distribution, percentage, mean, and standard deviation will be employed. Comparative analysis will be done to compare the antibiotic resistance characteristics in the two groups. Chi-square and other relevant statistical methods will be used to establish relationships between variables, where statistical significance will be determined at a $p < 0.05$ level [16].

Results

The following section highlights the results of the study on the isolation and immunological characterization of antibiotic-resistant Escherichia coli strains isolated from both human and animal sources in Libya. The results comprise information on the sample distribution, drug resistance patterns, MDR profile, and immunological characterization of the strains, as well as their statistical comparisons between human and animal strains.

Table 1. Distribution of Escherichia coli Isolates According to Sample Source

Source	Number of Samples	Percentage (%)
Human Samples	50	50.0
Animal Samples	50	50.0
Total	100	100.0

Distribution of the frequencies of E. coli isolates based on sample source is presented in Table 1. As can be observed in the findings of the study, a total of 50 (50.0%) human samples and 50 (50.0%) animal samples were collected. Considering the fact that both sources of samples had similar representation in the study, it can be stated that this study was quite representative in both cases.

Table 2. Distribution of Human and Animal Samples by Sample Type

Sample Type	Frequency	Percentage (%)
Human		
Urine	20	40.0
Stool	18	36.0
Wound Swab	12	24.0
Total	50	100.0
Animal		
Fecal Samples	22	44.0
Raw Milk	15	30.0
Meat Samples	13	26.0
Total	50	100.0

The number of samples categorized into animal and human samples is presented in Table 2. In human samples, urine samples constituted the largest number (20 samples or 40.0%), followed by stool samples (18 samples or 36.0%), with wound swab samples being 12 samples or 24.0%. The preponderance of urinary samples implies that UTI was likely to be the main source of isolation of E. coli from humans. The dominance of urine samples implied that human UTI was most likely responsible for isolating E. coli. In relation to the samples taken from animals, fecal samples constituted the highest percentage (22 samples or 44.0%).

The resistance pattern of E. coli bacteria against different antibiotics prescribed to patients is presented in Table 3. The maximum antibiotic resistance was observed in the case of ampicillin, where the total number of resistant isolates was found to be 68 (68.0%). A high resistance pattern was also seen in the case of tetracycline and cephalosporin with a value of 61% and 57%, respectively. Similarly, ciprofloxacin possessed a resistance pattern of 54.0%, whereas gentamicin possessed the least percentage of antibiotic resistance (42.0%).

Table 3. Antibiotic Resistance Pattern of *E. coli* Isolates

Antibiotic	Resistant n (%)	Sensitive n (%)
Ampicillin	68 (68.0)	32 (32.0)
Tetracycline	61 (61.0)	39 (39.0)
Ciprofloxacin	54 (54.0)	46 (46.0)
Gentamicin	42 (42.0)	58 (58.0)
Cephalosporins	57 (57.0)	43 (43.0)

MDR profile of the *E. coli* isolates is presented below in Table 4. As shown in the data, a total of 63 isolates, or 63.0% of all the isolates, proved to be MDR-positive, while 37 isolates were MDR-negative, which accounted for 37.0% of the total number of isolates. The resistant strains of *E. coli* in Libya are threatening public health in view of their high percentage of MDR positivity.

Table 4. Multidrug Resistance (MDR) Status of *E. coli* Isolates

MDR Status	Frequency	Percentage (%)
MDR Positive	63	63.0
MDR Negative	37	37.0
Total	100	100.0

The immunological properties of the *E. coli* isolates are given in Table 5. In all of the *E. coli* isolates, there are 58 isolates that possess favorable immunological properties, while there are 42 isolates whose immunological properties are negative. This indicates that these organisms contain virulence antigens, and this increases their pathogenicity.

Table 5. Immunological Characterization Results of *E. coli* Isolates

Immunological Result	Frequency	Percentage (%)
Positive	58	58.0
Negative	42	42.0
Total	100	100.0

The relationship between the presence of MDR among isolates of human and animal origins is shown in Table 6. In the human group, 34 isolates (68.0%) exhibited the presence of MDR while, for the animal group, 29 isolates (58.0%) had positive MDR. Isolates obtained from humans had a higher incidence of being multidrug resistant than isolates derived from animals. Nevertheless, there was the presence of MDR even among the isolates of these two sources, hence meaning that they might be carriers of MDR bacteria.

Table 6. Comparison of MDR *E. coli* Between Human and Animal Sources

Source	MDR Positive n (%)	MDR Negative n (%)	Total
Human Samples	34 (68.0)	16 (32.0)	50
Animal Samples	29 (58.0)	21 (42.0)	50
Total	63 (63.0)	37 (37.0)	100

Table 7 shows the distribution of samples based on animal species. Cattle contributed the highest number of samples (16 samples; 32.0%), while the other species included sheep and poultry (12 samples; 24.0%) and goats (10 samples; 20.0%). *E. coli* resistance would have occurred due to exposure of the cattle to the drugs more than other animals.

Table 7. Distribution of Animal Sources by Animal Type

Animal Type	Frequency	Percentage (%)
Cattle	16	32.0
Sheep	12	24.0
Goat	10	20.0
Poultry	12	24.0
Total	50	100.0

Chi-square test was employed for the comparison of the levels of resistance to antibiotics between humans and animals as shown in Table 8 below. Statistically significant differences were observed in the levels of resistance to Ampicillin ($p=0.042$) and Cephalosporins ($p=0.039$), whereby the human samples exhibited higher resistance compared to the animal isolates. There were no statistically significant differences in resistance levels to Gentamicin, Ciprofloxacin, and Tetracycline ($p>0.05$). It is thus clear that while some antibiotics show similar levels of resistance in humans and animals, some may show a strong correlation with exposure in clinical settings.

Table 8. Comparative Analysis of Antibiotic Resistance Between Human and Animal Isolates

Antibiotic	Human Resistant n (%)	Animal Resistant n (%)	χ^2 value	p-value	Interpretation
Ampicillin	38 (76.0)	30 (60.0)	4.12	0.042	Significant
Tetracycline	34 (68.0)	27 (54.0)	2.31	0.128	Not Significant
Ciprofloxacin	31 (62.0)	23 (46.0)	3.01	0.083	Not Significant
Gentamicin	24 (48.0)	18 (36.0)	1.52	0.217	Not Significant
Cephalosporins	33 (66.0)	24 (48.0)	4.25	0.039	Significant

The connection between the source of the sample and multidrug resistance (MDR) is shown in Table 9. Even though the prevalence of MDR was relatively higher among human isolates (68.0%) than that among animal isolates (58.0%), there was no statistically significant relationship between these two factors based on the results of the Chi-square test conducted at a p-value of 0.298.

Table 9. Association Between Source of Isolates and Multidrug Resistance (MDR)

Source	MDR Positive n (%)	MDR Negative n (%)	χ^2 value	p-value	Interpretation
Human	34 (68.0)	16 (32.0)			
Animal	29 (58.0)	21 (42.0)	1.08	0.298	Not Significant
Total	63 (63.0)	37 (37.0)			

In Table 10 is illustrated the relation between immunology outcomes and the origin of the isolates. There was more positivity for immunological outcomes among human isolates (64.0%) than among animal isolates (52.0%), but it was not statistically significant ($p=0.225$). Based on this, we can see that there are immunological features among both isolates which can suggest a link between them.

Table 10. Association Between Immunological Results and Source of Isolates

Source	Immunological Positive n (%)	Immunological Negative n (%)	χ^2 value	p-value	Interpretation
Human	32 (64.0)	18 (36.0)			
Animal	26 (52.0)	24 (48.0)	1.47	0.225	Not Significant
Total	58 (58.0)	42 (42.0)			

Discussion

There has been successful isolation of antibiotic-resistant *Escherichia coli* from both human and animal sources within Libya. The proportionate number of isolates in each sample is similar for each of the two groups of individuals. This

has made comparison between human and animal isolates more credible. From the human population studied, the greatest number of *E. coli* was obtained from the urine samples. This means that *E. coli* still accounts for many cases of UTI among humans. In agreement with this observation, other researchers have indicated that *E. coli* is the main cause of UTIs among humans [17].

In animal samples, the isolation rate was highest in fecal samples, indicating the role played by the digestive tract of animals in being a source for resistant organisms. It is quite evident that the existence of *E. coli* in raw milk and raw meat samples suggests that there could be a chance of transfer from the foods to humans. It has also been revealed through research that contact between animals, humans, and foods may cause such bacteria to transmit. It can be stated that the results obtained have confirmed the One Health theory. This theory implies that human health and animal health are interlinked when it comes to the spread of resistant organisms. Hence, the surveillance of *E. coli* in both populations is recommended [18].

From the results of the present study, there was observed high resistance of *E. coli* to antibiotic treatment in cases involving human and animal isolates. Resistance to ampicillin was found to be the greatest, followed by tetracycline and cephalosporins, whereas the minimum level of resistance was observed for gentamicin. Resistance to ampicillin is due to extensive use of β -lactam antibiotics among human beings and animals. Reports about such patterns have been made by researchers in various developing countries where antibiotics are easily accessible without any strict prescription regulations [19]. The emergence of ciprofloxacin and cephalosporin resistance is especially concerning since these drugs are used extensively in treating serious bacterial infections. The discovery of resistant strains of bacteria both among animals and humans indicates concern over possible transfer of resistant genes through the environment or food. These results show the growing prevalence of drug resistance in Libya and emphasize the need to adopt prudent use of antibiotics. Additionally, further research should be conducted to test resistance of bacteria to different antibiotics to help formulate appropriate strategies for resistance prevention. It can also be seen that inappropriate use of antibiotics in medicine and agriculture has been one of the main causes of antibiotic resistance [20].

The study revealed that a majority of the isolates were MDR positive because more than half of the isolates that were successfully isolated were found to be multidrug-resistant. Therefore, the findings revealed that there was resistance to several categories of antibiotics among the bacterial strains, which could make disease control difficult. The occurrence of the MDR bacteria may be associated with increased use of antibiotics, self-medication, lack of proper stewardship of antimicrobials, and improper use of antibiotics in livestock farms. There was a slight difference between the MDR levels of human and animal isolates, but the difference was not statistically significant [21]. This implies that the MDR strains of *E. coli* can be prevalent among the animal and human populations as well and their transmission could be taking place via similar modes of transmission. Moreover, considering the prevalence of MDR strains of bacteria in food products such as milk and meat highlights their role in causing zoonosis as well. Similar observations in other parts of the world have associated multidrug resistance in bacteria with increased durations of hospital stay, higher medical expenses, and even mortality. These results have emphasized the need for developing more stringent antibiotic control strategies and enhancing practices related to infections prevention among both humans and animals [22].

Based on the findings of immunological characterization, it was found that almost all *E. coli* isolates were immunologically positive to virulence-related and pathogenic-related antigens. This finding shows that many resistant isolates have virulence-related antigens that might enable them to be more virulent both in humans and animals. There was only a slight difference in the positivity rate of human and animal isolates in terms of their immunological response; however, there was no statistically significant difference between them. The similarities in the two categories could suggest some kind of connection in the epidemiology of the pathogenic bacteria. It is worrisome that the presence of virulence along with resistance poses difficulties in controlling infections by these bacteria [23].

Resistant and virulent strains can withstand antimicrobial therapy without impairing their capacity to infect host tissue. From the current study, results have further confirmed the findings from other studies concerning the role of certain strains of *E. coli* from animals, which may act as a source of zoonotic infections. Furthermore, the results indicate that there is a need for a multidisciplinary approach for investigating resistance among bacteria using microbiology and immunology approaches. Undoubtedly, a multidisciplinary approach provides much insight on pathogenesis and transmission processes associated with resistant bacteria. Therefore, more molecular studies should be carried out to identify virulent factors and genes associated with resistant *E. coli* strains in Libya [24].

It was found that there were statistically significant differences regarding resistance to ampicillin and cephalosporins

between human and animal isolates but no statistically significant differences with regard to tetracycline, ciprofloxacin, and gentamicin resistance. The fact that human isolates were more resistant might be associated with greater use of antibiotics among humans due to the prescription of these drugs in clinics as well as their misuse. At the same time, the lack of statistically significant differences for some of the antibiotics indicates that resistant strains might be transmitted from humans to animals and vice versa. Furthermore, the statistical analysis failed to find a link between the resistance of the isolates to multiple antibiotics and their immunological positivity. Both humans and animals contribute to the prevalence of resistant strains in the environment [25]. The lack of marked variation in some variables also serves to increase the probability that these pathogens are likely to exhibit common mechanisms of resistance and modes of transmission. This observation adheres to the One Health approach since the importance of considering the interplay between human, animal, and environmental components in the emergence of antimicrobial resistance is recognized under this concept. It becomes clear that the significance of adopting a holistic public health strategy, involving the participation of health workers, veterinarians, food inspectors, among others, can be derived from this research [26].

Conclusion

This research was successful in isolating and characterizing the immune system properties of antibiotic-resistant *Escherichia coli* isolates in both humans and animals in Libya. The results indicate a high occurrence of antibiotic-resistant isolates of which a large number are resistant to ampicillin, tetracycline and cephalosporins. There was also a relatively high percentage of isolates that were found to be multidrug resistant isolates which is indicative of the growing threat of resistant *E. coli* isolates to human health. The immune system characterization of the resistant isolates revealed virulence features for some of the isolates. Comparative study of resistant isolates obtained from both humans and animals revealed similarity in resistant patterns hence making transmission of *E. coli* isolates possible from humans to animals and vice versa through either contact or contaminated food products. In accordance with the findings of this study, it is recommended that greater attention should be paid to promoting better programs related to antibiotics. There needs to be proper surveillance and monitoring of antibiotic resistance pathogens at hospitals, farms, and food processing facilities. There must also be initiatives to create awareness about the dangers of abusing antibiotics. Finally, it is recommended that studies be done on a molecular basis to understand resistance and pathogenic genes in *E. coli* bacteria in Libya.

Conflict of interest. Nil

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