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A Molecular Detection for Some Bacterial Resistance Genes *Escherichia Coli* Isolated from Fresh Red Meat in City of Al- Nasiriyah

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Abstract:
The present study was conducted to detect the contaminated microbial pathogens of fresh red meat in different places of the al-butcher shops, where 100 samples and swabs were collected from different places and tools from meat shops in different areas in Nasiriyah for the period from September 2018 to March 2019. The highest isolation rate for *Escherichia coli* isolates was different according to the type of sample taken (31.53% meat samples, 21.42% the ground, 21.33% cutting boards, 19.1% cutting tools). 15 isolates were selected for the sensitivity test for eight antibiotics, to detect resistance genes, *sul1* and *tet (B)* The isolates were highly resistant to Ampicillin, Vancomicin, Amoxicillin at a rate of 100%, they were very sensitive to antimicrobial antiperspirants such as Trimethoprim, Norfloxacin, Chloramphenicol at a rate of 100%, The sensitivity of Tetracycline and Gentamycin was 86.7% and 80% respectively. The results of the electrical relay were shown after DNA extraction using Kit and amplified the genes using a PCR. Specialized gene primers were used for each gene, the presence of the resistant gene *sul1* in the isolates of *E. coli* was 80%, and the *tet (B)* was 87.3%. Some isolates were record as new strains in the NCBI gene bank database.

Keywords: Red meat contamination, *Escherichia coli*, Sensitivity test, Resistance genes.

1.Introduction:

Meat is one of the basic and important foods for human food because it contains a high proportion of proteins, fats and mineral elements such as phosphorus and iron in addition to large quantities of vitamins B, which are good sources needed by the body to carry out the metabolic processes and various vital events. In addition, meat is a food that causes many human health problems, and this occurs when health conditions are not available in slaughter houses or through marketing and trading [1]. Meat is one of the most common causes of food poisoning in many countries where meat consumption is high. Cases of microbial toxicity in America were estimated at about 70% -60%, with the main culprit being contaminated meat [2]. There are several sources that lead to meat contamination including water, air, soil, feed, feces, intestines, leather, equipment and processing utensils as well as slaughter workers. All these factors lead to contamination of healthy animal muscles during slaughter processing and processing [3]. and there are several factors that cause contamination of meat may be external, such as the presence

or absence of oxygen and temperature and internal factors such as pH, amount of moisture and nutritional value of meat in terms of their containment of proteins, sugars and fats [4-6].

Beef sold at the al- butcher shops is subject to a series of slaughter transport and treatment, and each of these stages causes the contamination of the meat with many microbes [7]. The quality of the microorganisms that cause meat contamination is linked to the physiological state of the animal at slaughter as well as to transport storage and storage. The meat surface colonizes many macrophages through the various stages of meat preparation [5]. Meat contamination is achieved through the general census of the microorganisms located up to 10^7-10^8 cm² of meat [8]. One of the most common bacterial species found on fresh meat is (*Acinetobacter, Psedomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, Micrococcus*, *Lactic acid* bacterium and other enterobacteriaceae, including *Salmonella spp, Escherichia coli, Klebsiella spp, Proteus* [9][10]. Meat of all varieties including beef is the main store of colonic bacteria Which is responsible for the contamination of meat and meat products E. *coli* is an intestinal bacteria isolated from the stools of infants by Escherich for the first time in 1885 [11]. These bacteria are normal flora where these bacteria live in the human intestines and mammals including cows [12]. These bacteria cause many diseases for humans They cause gastroenteritis in infants, meningitis, Bacterimia and Pneumonia. They also play a role in wound injury [13] also causes urinary tract infection, Cystitis, pyelone purities [14]. The resistance to antibiotics in bacteria is one of the biggest and most important problems facing society. The repeated, indiscriminate and sometimes non-aggressive use of antibiotics in human and veterinary medicine and other uses has resulted in the spread and development of resistance in bacteria not only in human and animal isolates but also in the environment [15]. The resistance to antimicrobials especially among family members is a major threat to public health. The resistance of bacteria to antimicrobials is natural or acquired, Natural resistance depends on the physiology and genetic properties of the bacteria or on the composition of the organism. Biochemistry and its mechanism of action. The acquired resistance depends on the genetic mutations that cause the emergence of resistance genes that pass through generations and strains of the same species or between different types within the family or through the acquisition of resistance genes from bacteria to other bacteria [16]. Genes are transmitted between races by the presence of plasmids, which are important vectors of genes, which are responsible for demonstrating multi-resistance to antibiotics [17]. Plasmids are DNA rings outside the chromosome that have the ability to replicate independently of the host cell chromosome, which encodes a wide range of functions such as the production of external toxins, antibiotic resistance and heavy metals [18]. The production of broad spectrum βeta-lactmase enzymes (ESBLs) is an important mechanism that helps the bacterial cell to resist these enzymes are complex, diverse, and rapidly evolving and pose a threat to many antibiotics [19]. Bacterial cell exposure to βeta-lactamase leads to the production of βeta-lactamase enzymes continuously, which leads to genetic mutations in the genes encoding them, causing the emergence of coding resistance genes to the enzymes beta-lactamase broad-spectrum, which gives the resistance to the bacterial cell towards many βeta-lactam antagonists such as Penicillins and Cephalosporins [20].

Aim of the study

Isolation and diagnosis of the most important pathogenic bacterial species contaminated with freshred meat from al-butcher shops in city of Al- Nasiriyah, Demonstrate the resistance of some isolated bacterial species to some antibiotics, Molecular detection of a number of antibiotic resistance genes for some isolated bacterial species.

Material and Methods:

a. Samples and Collection:

A total of 100 samples of fresh beef were collected from various locations in al-butcher shops for a period of six months starting from September 2018 to March 2019. They were collected from different areas of meat shops in city of Al- Nasiriyah, at a rate of 3 random shops with 25 g for each sample after the

application of the possible purification and sterilization procedures. The samples were taken after being placed in clean, sterilized bags of polyethylene, and the swabs were taken by the carrier's stem medium. and the number and the name of the sample and the location and date of withdrawal of the sample in a refrigerated manner using a container on the ice, to the Microbiology Laboratory of the Department of Biology/ College of Education Sciences Pure / University of Thi-Qar to reveal some pathogenic bacteria that may be present on the red meat.

b. Samples Culture:

One gram of fresh beef taked and cut into very small pieces. Wipes are placed directly in a sterile 10 ml tube containing 9 ml of Peptone Water solution,1ml transported to another tube containing 9 ml of sterilized distilled water, and a series of decimal dilutions was administered from 10^1-10^4 and 0.5 ml was taken by a sterile pipette to a Petri dish containing 20 ml from the nutrient agar medium Himedia (India). It was inoculated on the surface of the agricultural medium by the method of homogeneous diffusion using an L-shaped glass rod and then incubated at 37 C° for 24 hours for the purpose of investigating some types of pathogenic bacteria and the number of colonies [21].

c. Diagnosis of Bacterial isolates:

The isolates were diagnosed through phenotypic diagnosis, Gram stain, biochemical test, Vitek2 diagnosis, molecular diagnosis of PCR using a number of antibiotic resistance genes, bacterial isolates were identified by studying the general vegetative properties of the developing colonies on nutrient agar, Eosin Methylene Blue agar, MacConeky agar, Blood agar Himedia (India). The colonies were then studied, their colors, their size, the colon of the colonies, as well as their ability to decompose the blood on the blood agar. Bacterial isolates were subjected to microscopic examination Several cultivars were prepared for each bacterial farm and were labeled Gram Stain and were examined under the 100 x magnification lens to monitor bacterial cell response shape and order [22].

d. Antibiotic Susceptibility testing of isolation:

The method of spreading the tablets was used to detect resistance to eight antibiotics. The areas of inhibition were compared to the table of the diameters of Inhibition Zones used to determine the resistance or sensitivity of isolates of antibiotics according to the guide lines of the clinical and laboratory standards instiute [23].

e. DNA Extraction:

Total DNA was extracted for bacterial isolates to use in molecular diagnostics Several Purification Kit processed by Promega USA were used. It was then electrolygated on 1.5% agarose gel and with effort 75 volts for 45 minutes after dyeing the safe dye green and examining the gel by exposing it to UV source by UV-Transilluminator and direct images

f. Polymerase chain reaction:

PCR was used to amplify DNA as well as to investigate antibiotic resistance genes, using the primers shown in Table (1) [24] [25].

Table (1): Sequence of primers used to diagnose resistance genes and gene size

µl A 20

The gene		Primer sequence	gene size pb	Program	Number of courses
sull	F	TTCGGCATTCTGAATCTCAC	822	95°C\15m 94°C\30s 58°C\1m 72°C\10m 72°C\1m	35
	R	ATGATCTAACCCTCGGTCTC			
tet(B)	F	GGTTCACTCGAACGACGTCA	634	95°C\3m 94°C\30s 57°C\1m 72°C\10m 72°C\1m	35
	R	CTGTCCGACAAGTTGGATGA			

polymerization reaction was carried out based on the attached kit from Bioneer, Korea

Follow the program shown in Table (2) to amplify DNA samples using PCR [26].

Table (2): PCR DNA amplification program

Stage	**Steps**	**Temperature**	**Time (minute)**	**Number of cycles**
The first	Initial denaturation	94	3	1 cycle
The second	Denaturation 2	94	30	32 cycle
	Annealing	56	30	
	Extention 1	72	1.5	
The third	Extention 2	72	10	1 cycle

The electrophoresis replication products of the samples were detected on the agarose gel and the DNA Ladder (2000bp) solution in the first hole of the gel for the diagnosis of bacterial isolates and to locate the antibiotic resistant genes in the passed DNA, Completion of the transfer The samples were examined under ultraviolet light to see the beams and then photographed directly with the camera.

g. Statistical Analysis:
The results of the study were statistically analyzed using (SPSS) at a probability level ($P \leq 0.05$).

3- Resulte:
a. Isolation and diagnosis of contaminated bacteria of red meat:
The bacterial isolates were examined after phenotypic tests, biochemical tests, Vitek2 compact tests and molecular tests. The table (3) shows that the highest isolates were *E.coli* and isolates of meat 31.53%, Followed by the isolation of bacteria from the survey from the ground 21.7%, and the lowest percentage of the survey was obtained from knives and cutting tools 19.71%, while the percentage of isolating bacteria from samples taken from cutting plates 21.33%. The table (4) shows Statistical analysis of numbers and percentages of isolated bacterial species.

Table (3): Numbers and percentages of isolated bacterial species

Bacteria	Meat	%	Cutting boards	%	Tools cutting	%	Ground	%
E.coli	35	31.53	16	21.33	14	19.1	15	21.42
K.pnemoniae	23	20.72	14	18.66	13	18.30	14	20
En.aerogenosa	10	9	8	10.66	10	14.08	6	8.57
Ps.aerogenosa	8	7.20	7	9.33	8	11.26	8	11.42
Staph.aureus	6	5.40	7	9.33	6	8.45	7	10
Citro.freundii	4	3.60	5	6.66	4	5.63	4	5.71
Other	25	22.52	18	24	16	25.35	16	22.85
Total	111	100	75	100	71	100	70	100

Chi-Square Tests	Value	*df*	Asymptotic Significance (2-sided)
Pearson Chi-Square	9.742[a]	18	.940
Likelihood Ratio	9.667	18	.942
Linear-by-Linear Association	1.666	1	.197
N of Valid Cases	403		

a. 0cells (0.0%) have expected count less than 5. The minimum expected count is 5.71.

$X^2 = 9.742$ $df = 6$ P-Value = 0.94

b. Antibiotics Sensitivity Test:
The sensitivity test for bacterial isolates was tested for eight common antibiotics. 15 isolates of *E. coli* towards eight types of ready-made antibiotics and in the Disc Diffusion Method. The table (5) shows sensitivity and resistance to bacterial isolates against the used antibiotics. *E. coli* isolates showed high

resistance to the antibiotics Ampicillin, Vancomicin, Amoxicillin at a rate of 100%, and they were very sensitive to Trimethoprim, Norfloxacin, Chloramphenicol at a rate of 100%, the sensitivity of Tetracycline and Gentamycin was 86.7%, 80% Respectively. The table (6) shows Statistical analysis of the sensitivity and resistance of E. *coli* bacteria to antibiotics

Table (5): Sensitivity and resistance to antibiotics

Antibiotic	Disc content (mg)	Resistance	%	Sensitivity	%
Tetracycline	25	2	13.33%	13	86.7%
Ampicillin	10	15	100%	0	0
Gentamycin	30	3	20%	12	80%
Vancomicin	25	15	100%	0	0
Trimethoprim	10	0	0	15	100%
Amoxicillin	30	15	100%	0	0
Norfloxacin	10	0	0	15	100%
Chloramphenicol	10	0	0	15	100%

Table (6): Statistical analysis of the sensitivity and resistance of E. *coli* bacteria to antibiotics

Chi-Square Tests	Value	*df*	Asymptotic Significance (2-sided)
Pearson Chi-Square	687.607[a]	7	.000
Likelihood Ratio	909.127	7	.000
Linear-by-Linear Association	49.764	1	.000
N of Valid Cases	800		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 41.63.

X^2 =687.60 *df* =7 P-valu
e=0.00

c. Detection of *sul1and tet(B)* genes resistance to antibiotics using PCR

15 isolates were selected to detect the presence of antibiotic resistance genes. The results showed that there was a *sul1* gene in 12 isolates at an average rate of 80%, while a *tet(B)* was found in 13 isolates with an average of 87.3% as shown in the Table (7), Figure (1 and 2) shows the results of the electrophoresis of the gene *sul1and tet(B)*.

Table (7): Numbers and percentages of the presence of Antibiotic resistance genes

Resistance gene	Positive	%	Negative	%
sul1	12	80%	3	20%
tet(B)	13	87.3%	2	13.3%

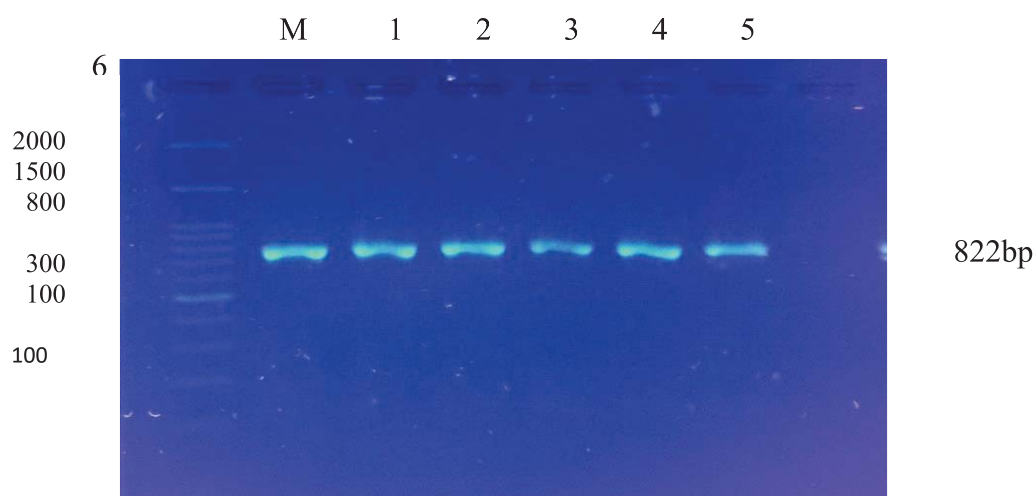

Figure (1): Electrophoresis on the gel of agarose with a 1.5% concentration of *sul1* resistant sulfonamide.

•Marker (M) The measured gauge weighs (100-2000 pb).
•Gene bundles (*sul1*) in all isolates and the weight index (822pb).

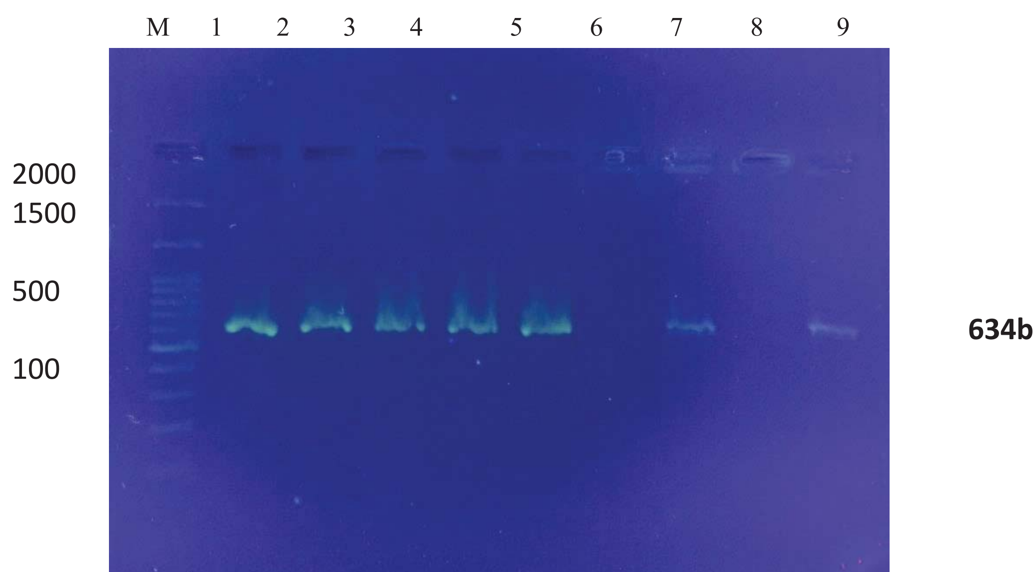

Figure (2): Electrophoresis of the agarose gel with a 1.5% concentration of *tet(B)* resistant tetracycline.

•Marker (M) The measured gauge weighs (100 -2000 pb)
. The gene (*tet(B)*) in all isolates and the index weighed (634pb).
• Isolated (6 and 8) are negative for the gene.

d. DNA Sequencing:

DNA Sequencing analysis of *E. coli* isolates was performed using the BLAST alignment tool (Blastn). The homogeneity of studied isolates was determined with the closest isolates recorded in the gene bank data, based on the highest match and best ratio and the lowest E-value. The nucleotide sequence of most isolates was processed using the FinchT.V program for pruning undesirable low-sequence rules (base density less than 20%). The results of the present study showed that isolates gave a positive result to DNA

sequencing of resistance genes by comparing them with The bacterial species recorded in the gene bank data, where Three new strains of *E. coli* were recorded, with ratios ranging 99% with other isolates and recorded in the NCBI gene bank, and their own codes were given with 3 bacterial strains (HR1, HR2, HR3) NCBI gene data. The table (8) shows New strains recorded in the current study with the *E. coli* Gene Bank.

Strains of Study	Accession number	ID %	Country	Match Accession Number	Variation			Range
					Location	Substation	Type	
Escherichia coli, Strain HR1	MN095194	99	Australia	MK599281	32065 32593 32629	---G ---T A-G A--T	Insertion Insertion Transition transversion	: 32051 to 32645
Escherichia coli, Strain HR2	MN095195	99	USA	-CP026206 135/136(9	111499 111515	G-A T-A	Transion transversion	:111478 to 112060
Escherichia coli, Strain HR3	MN265384	99	Japan	CP038455	7392	---G	Insertion	7367 to 8094

Plus/Plus

4- Discussion

The results of this study showed the isolation and diagnosis of several types of bacteria and the dominance of *E.coli* bacteria contaminated with red meat in various samples taken from the shops of the butcher and the results of this study are close to study [27], where their study showed the dominance of colon bacteria in local and imported red meat, The results also converged with [28] confirming that 80% of the chicken meat samples and 50% of the samples of the pig meat had the predominance of colonic bacteria in these samples, especially *E.coli* who indicated that Fecal contamination can not be prevented during the different stages of the production process. The supply and slaughter tools also contribute to increasing the percentage of *E. coli* contamination in calf's meat, due to the lack of hygiene in the qasabah and slaughterhouses [29], and the results were consistent with the researcher [30] for *E. coli* and different isolates. The results of the study differed with [31] where their results indicated the prevalence of Staphylococcaceae, the isolation ratios of *E. coli* were low (1.48%), This may be due to the cleaning of the meat after the removal of the intestines and the cleanliness of the workers in the slaughterhouses and stalls. The isolation rate was low for the researcher's study [32] where the isolation rate was *E. coli* 15.7%. whereas for the cutting boards the results were somewhat similar with [33], the presence of different types of bacteria on the surfaces of these plates may be due to the fact that Washing and keeping these plates is not good, and the use of these panels cause pollution, as well as the growth and multiplication of many of the bacteria on their surfaces, and this leads to the transfer of microbes to humans, causing many diseases such as blood vessels and convulsions and other [34], and did not agree with the results of the study with

the study of [35] Where the rates of isolation of bacteria *E.coli* they have 4.11% and may be attributed to the low rate of isolation to hygiene and health conditions, and also noted that each of the [36] researchers during their study on 90 samples of beef that the colon infection is mainly on the surfaces of meat. This is attributed to weakness and lack of adherence to health conditions in the massacres, as well as the circulation of meat, causing health problems and a significant risk to human health. The survival of meat for different periods in al-butcher shops in different areas and in varying degrees is one of the reasons for pollution [37]. External sources such as contaminated knives, transport vehicles, land and workers' hands may cause pathogens to enter the flesh during slaughter, blood thinning [38], He pointed out [39] that the increase in the number of bacterial isolates and their diversity in meat indicates that the low quality bacteriological, which makes them a source of food infection, and the different health conditions in the al-butcher shops addition to the unhealthy methods followed by The differences in the isolation rates of different bacterial species among the different regions of Iraq as well as the countries of the world may be attributed to the extent to which health procedures are applied everywhere, as well as the development of the country's technology and its aftermath, and the nature and intensity of production.
One of the most serious problems facing the world from the medical point of view is the acquisition of bacterial isolates to the degree of resistance to antibiotics, resulting in the difficulty in choosing the appropriate treatment for patients. The indiscriminate use of antibiotics without a sensitivity test makes bacteria able to adapt and thus increase their resistance to antibiotics used for treatment [40]. The results of the present study showed a difference between bacterial isolates in their antibiotic resistance *E. coli* isolates showed high resistance to Ampicillin, Vancomicin, Amoxicillin at a rate of 100%, and were very sensitive to Trimethoprim, Norfloxacin, Chloramphenicol at a rate of 100%, Its sensitivity to Tetracycline and Gentamycin was 86.7% and 80%, respectively. The results of the study differed with [41]. The resistance ratios for Ampicillin and Tetracycline were 50%, while the results were close to their sensitivity to Gentamycin, Trimethoprim, Chloramphenicol at a rate of 100%, 75% and 50%, respectively. The results were also different with the study of [42], for the antibiotic Amoxicillin, where the bacteria were very sensitive to him and 100%. For Gentamycin and Trimethoprim, the results were close with sensitivity of 100% and 71.43%, respectively. The results were compared with [43] for Ampicillin and Amoxicillin with 65.7% and 66.67% respectively. The sensitivity of Tetracycline and Chloramphenicol was 51.4% and 74.3% respectively. A [44] fold study showed variation in resistance and sensitivity of isolates with isolates resistant to Vancomicin at 88.89%, while isolates were sensitive to Amoxicillin, Trimethoprim, Gentamycin and 86.67%, 82.22% and 75.56%, respectively. There were also [45] high resistance ratios for isolates for both minced meat and beef 67.5%. This discrepancy is due to the antibiotic resistance ratios of the *E. coli* isolates for their acquisition of β- Lactamase enzymes, which has been able to resist various antimicrobials [46] [47]. The resistance of bacterial isolates to antibiotics is due to several reasons, including the containment of certain enzymes encoded genes such as β-Lactmase enzymes, which destroy resistant antiboiotics or bacteria acquire pumps, which in turn contribute to the destruction of antimicrobial agent of the cell before the antibody reaches the target site and performance Currency, or bacteria acquire from the metabolic pathway many genes, and thus a change in the bacterial cell wall will occur, or bacteria will acquire a genetic mutation that prevents the antimicrobial agent from reaching the target site within cell [48]. The indiscriminate use of most antibiotics in communities and hospitals has led to the emergence of strains resistant to many antibiotics and thus the inefficiency of treatment, which is one of the most important problems from the medical point of view due to lack of appropriate treatment for the patient [49], To counteract the risk of bacterial resistance to highly used antibiotics, the efforts of

all segments of society - doctors, pharmacists, nurses and patients - must be combined to reduce the spread of antibiotic-resistant bacteria.

The global trend in the last decade to use DNA sequencing has increased because of its ability to rapidly diagnose species and bacterial species far better than those of conventional methods of diagnosis [50]. DNA sequencing is also an important and useful technique in Knowledge of the relationships between bacterial species, thus becoming the best standard for the diagnosis of species and bacterial species. This technique was used to diagnose up to 90 thousand bacterial strains. Where the current trend is to combine phenotypic and genetic diagnosis [51]. The molecular study included the extraction of DNA using Kit, and antibiotic-resistant sul1 and *tet(B)* genes were also used. The results of the study showed that the rate of the presence of the gene *sul1* was 80%, and the rate of the presence of the gene *tet(B)* 87.3% of the total isolation of 15 *E. coli* the results of the study [20]. Were found in isolates with 89.5% while sul1 was found to be less than 39.5%, and the presence of the *tet(B)* gene in [52] Less than our study and by 41.3%. The study showed that 3 new antibiotic-resistant strains with ratios of 99% with other isolates were recorded in the NCBI gene bank and were given their own code named 3 strains of bacteria (HR1, HR2, HR3) and are available in the gene bank database NCBI.

5-Conclusions:

1. The general environment and the environment of slaughter houses and environments where meat sales play a big role in the contamination of fresh meat, because these environments are rich in many microorganisms of different races and species.
2. Contamination of meat with genera and types of microbes after slaughtering animals in slaughterhouses and al-butcher shops during skinning and cutting and transport and manual handling and lack of cleanliness of workers, as well as contamination of cutting tools and lack of hygiene and lack of health conditions.
3. Contamination of meat in al-butcher shops in the city of Al- Nasiriyah several bacterial species.
4. The random description of antibiotics by some doctors and pharmacists and the failure to do an allergy test to the emergence of bacterial strains resistant to antibiotics.
5. Increased use of antimicrobial agents in animal production as a means of promoting growth, preventing and treating diseases leads to the emergence of antibiotic-resistant bacterial strains. These antibiotic-resistant strains can be transmitted from animal to human through the food chain and thus food acts as a vector for resistant bacteria and genes. Resistance to man.

The use of molecular methods is a fast and effective way to detect genes resistant to antibiotics of pathogenic bacteria.

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