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Multidrug-Resistant Bacteria from Raw Chevon and Mutton Meat

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Abstract

ANTIMICROBIAL resistance is a major global issue for human and animals. Increased use of antimicrobials in livestock has become one of the causes of antimicrobial resistance development in foodborne bacteria. The aim of the present study was to characterize antimicrobial resistant bacteria, Escherichia coli and Staphylococcus aureus, from one-hundred raw chevon and mutton meats (50 of each) collected from random slaughterhouses in Qalubiya governorate by standard in vitro techniques according to Clinical & Laboratory Standards Institute (CLSI) guidelines. Out of 100 samples, E. coli was detected in 36% and 50%, while *S. aureus* was detected in 44% and 52% of the examined chevon and mutton meats, respectively revealing mutton to be more subjected to bacterial contamination than chevon. Results of different antibiotic challenging against the detected isolates, most of them showed high levels of resistance against different used antibiotics; where mutton's isolates showed higher resistance levels than chevon's isolates. The highest resistance ratios were reported against tetracycline, cefaclor and amoxicillin. While, they were almost sensitive to azithromycin, ceftriaxone and norfloxacin. Referring to the molecular identification of drug resistance genes, tetA and blaTEM were detected in all of the examined *E. coli* isolates; whereas, aada1 gene were detected in 80% of the examined isolates. On the other hand, mecA and tetk were detected in 80% of the examined *S. aureus* isolates, while vanA gene was detected in only 40% of the examined samples. Therefore, strict hygienic conditions and wise usage of antibiotics in live stocks are strongly recommended.

Keywords: Fresh meat, Multidrug resistance, Slaughterhouses,

Introduction

Meat is an essential part of our daily diet and a nutrient-dense food that has higher protein, vitamin, and mineral content than other food sources. It is also required for the growth, maintenance, and repair of body cells [1]. Nonetheless, according to EFSA [2], it is considered a major vector of human food-borne infections. Fresh meat's pH and water activity are important parameters for the growth of microorganisms. During handling, processing, preparation, and distribution, pathogenic bacteria can infect meat and meat products, which can have detrimental socioeconomic effects [3].

Among biological threats, consumers are particularly concerned about bacterial infections, as eating contaminated food can lead to hospitalization or even death in cases of mild to severe illness [4]. However, although exact statistics are unavailable, the incidence of food-borne illness seems to be higher in developed nations.

The primary sources of contamination in fresh carcass meat are thought to be soiled animal hide and hair, knives, hands, arms, worker clothing, and unintentional piercing of the gastrointestinal tract (GIT) during the skinning and evisceration process [5].

Enterobacteriaceae are a diverse group of organisms that are not exclusively derived from feces. They are commonly used as a gauge for the overall hygiene standards in the slaughterhouse [6]. Furthermore, the gastrointestinal tract is frequently a source of carcass contamination, as evidenced by the frequent isolation of enteric organisms from meat, such as E. coli [7]. Alternatively, there is S. aureus, a common foodborne pathogen. According to Rossi *et al.* [8], it is implicated in a number of infections that affect humans, including meningitis, food poisoning, bacteremia, urinary tract infections, pneumonia, and heart valve infections. By consuming food that has been produced with inadequate hygiene measures and/or improper storage procedures, they can infect food.

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These bacterial infections are regarded as a global health threat due to the introduction and rapid spread of multidrug-resistant bacteria (MDR) in humans, animals, and the environment. Globally, multidrug resistance is increasing and is regarded as a threat to public health. According to a number of recent studies [9, 10]. bacterial multidrug resistance (MDR) pathogens can emerge from both humans and animals.

According to the level of antibiotic consumption, which is determined and controlled by a nation's antibiotic policies, the pattern of antibiotic resistance differs between regions and countries [11]. Drug selection requires constant monitoring of antibiotic susceptibility due to the evolution of multidrug resistant strains.

The emergence of MDR bacteria from meat and meat products has been linked to several studies. It is often associated with antibiotic misuse that ends up being released into the environment, antibiotic residues (parent antibiotic or its metabolites or both found in animal derived products) in livestock products and wastes, and, last but not least, a lack of strict and efficient oversight and control over the production, use, and disposal of antibiotics [12].

According to Zerabruk *et al.* [13] the meat supply chain in developing nations lacks sufficient controls to guarantee microbial quality, safety, and hygiene. This includes butchers, slaughterhouses, distribution, and final consumers. Additionally, there is a dearth of information to evaluate the microbial load of meat contact surfaces in butcher shops and abattoirs as well as food safety procedures. Governments and other stakeholders may find it challenging to apply precise measures to the impact of food contamination issues on public health as a result of these factors [14].

Therefore, this study was designed to determine antimicrobial resistance patterns of some foodborne pathogens in raw freshly dressed goat and sheep meats in relation to abattoir hygiene and sanitary conditions.

Material and methods

Collection of samples

A total of 100 random samples of different raw, freshly dressed goat and sheep carcasses' meat cuts (50 of each) were collected from each carcass in various slaughterhouses located in Qalubiya government. Each sample was presented to the following steps for evaluation of their bacteriological quality:

Preparation of samples [15]

Twenty five grams of each sample were mixed with sterile peptone water (0.1%) and kept at 37^0Cfor 24h; from which the following parameters were examined:

Prevalence of Enteropathogenic

Escherichia coli was performed according to ISO 16649-2 [16]. included plating on Tryptone Bile X-glucoronide agar (TBX agar) followed by incubation at 44oC for 24h. Suspected colonies appeared Greenish-blue colonies were furtherly confirmed by biochemical identification. Strains were isolated and positive samples were recorded.

Prevalence of Staphylococcus aureus

It was performed according to ISO 6888- 1 [17]. by plating of the previously prepared samples on Baird Parker agar and incubated at 37oC for 24-48h. Suspected colonies, appeared as black colonies with halo margins, were purified and subjected for further biochemical identification. Strains were isolated and positive samples were recorded.

Antibiotic Sensitivity Test

In-Vitro sensitivity test was done on each isolated E. coli and S. aureus strains of each examined species to study their sensitivity for different antibiotics using the disc-diffusion method on Muller-Hinton agar and incubation for 24h in 37^OC according to CLSI [18]. Various types of antimicrobials related to beta-lactams, tetracycline, quinolones, macrolides and glycopeptide groups were evaluated against *E. coli* and *S. aureus* isolates separately.

Molecular detection of antibiotic-resistant genes

Five multidrug resistant (MDR) isolates of the confirmed *E. coli* and *S. aureus* strains, represented by 3 isolates from mutton meat and 2 isolates from chevon meat, were sent to the Central Laboratory for Veterinary Quality Control on Poultry Production (CLQP-PCR unit), Animal Health Research Institute for molecular detection of some antibiotic resistance genes represented by mecA, vanA and tetK for *S. aureus*, and blaTEM, tetA (A) and aada1 genes for *E. coli*.

Primer sequences of the chosen genes for *E. coli* and *S. aureus* used for PCR system following were mentioned in Table (1).

Percentage of antibiotic sensitive and resistance strains was calculated using the following equation:

$$\frac{No.of\ sensitive\ or\ resistant\ isolates}{Total\ No.of\ the\ isolates} x100$$

N.B. Antibiotic sensitivity profiles were presented as a percentage (%) calculated in relation between the number of the sensitive and resistant isolates to the total number of isolates.

Results

Referring to the recorded results in Tables (2), *E. coli* and *S. aureus* were detected in a total of 43% and 48% of the examined samples; where, mutton

meat samples showed higher contamination levels either with *E. coli* or *S. aureus* with incidences of 50% and 52%, respectively.

Results of different antibiotic susceptibility of *E. coli* isolates, most of them showed high levels of resistance profile against different used antibiotics; where chevon's isolates showed higher resistance levels than mutton's isolates. The highest resistance ratios were reported against tetracycline (80%) and doxycycline (72.0%) for mutton's isolates; and for cefaclor and tetracycline (83.3%) for chevon's isolates (Tables, 3 and 4).

Discussion

A major public health concern is the presence of drug residues and other substances in food products, especially in light of the increasing awareness of the emergence of antimicrobial resistance (AMR). Antibiotics are frequently administered subtherapeutically or prophylactically to prevent bacterial infections in food animals. This has resulted in the formation of multidrug-resistant bacteria, a major public health concern, along with the residue left behind. Antibiotic resistance has been observed in a number of microorganisms, leading to the emergence of new antibiotics with increased resistance [19].

Using too many antibiotics when raising livestock is one of the primary causes of AMR infection in humans. AMR infections that are resistant to the current antibiotic regimens cause more than 700,000 deaths annually. The two bacteria that cause AMR-related deaths most frequently are *S. aureus* and *E. coli* [20].

The recorded results of the present study regarding with the prevalence of *E. coli* and *S. aureus* in the examined samples, lower prevalence was recorded by Al-Asmari *et al.* [21]. (30%); while, Kim *et al.* [22]. recorded higher bacterial contamination in goat's meat than sheep samples. Whereas, it came in line with Thwala *et al.* [23]. who recorded positive detection of *S. aureus* in the examined samples with prevalence of 33.08%.

Variations between different authors may be attributed to differences in hygienic levels in their slaughterhouses, personal professionality and the sanitary conditions of the transportation and storage.

Poor hygiene during the evisceration and preparation of the dressed carcass leads to the contamination of fresh meat by *E. coli* and *S. aureus*; strains associated with humans and animals may be involved [24].

The United Nations 2030 Agenda states that ensuring food safety involves addressing AMR bacterial concerns. The primary cause of resistant bacteria in the food industry is improper and abusive use of antibiotics, which includes using them to stimulate animal growth [25].

Results of antimicrobial sensitivity test as summarized in Tables (2-5) were somewhat agreed with the results recorded by Herawati *et al.* [26], Barilli *et al.* [27]., Bahbah [28]. Hosny [29] who recorded that *E. coli* and *S. aureus* strains isolated from contaminated meat and meat products are resistant to commonly used antibiotics; multidrug resistance of their foodborne bacterial isolates detected in meat and meat products. In which, they recorded that the overall information indicated that the detected bacterial species from goats and sheep meat were resistant to penicillin, ampicillin, amoxicillin, chloramphenicol, streptomycin, tetracycline, cephalothin, gentamicin, ciprofloxacin and sulfamethoxazole. The prevalence of antibiotic resistance ranged from 0.4% to 100%. However, *S. aureus* and *E. coli* were highly resistant to all antibiotics tested.

Many drug-resistant genes are typically present in multidrug-resistant (MDR) bacteria. These genes can arise from gene mutations or horizontal gene transfer. Because it produces the β-lactamase enzyme, which breaks down the β-lactam ring of most penicillin derivatives, *E. coli* has developed resistance to β-lactam antibiotics. Additionally, the *mecA* gene, which codes for a modified penicillin-binding protein (PBP2a or PBP20) with a lower affinity for binding β-lactams, confers resistance to all β-lactam antibiotics on the majority of S. aureus isolates [30].

Regarding with the molecular study, the obtained results came in line with the recorded results of Naeim *et al.* [31] and Elboghdady *et al.* [32]. who recorded positive detection of various antibiotic resistance genes of different isolated bacterial strains collected from meat and meat products.

Conclusion

Chevon and mutton meat showed potentiality to be loaded with food pathogens as a consequence of inadequate hygienic measures during their dressing and preparation. Mutton meat, in the current study, had higher contamination level than chevon meat samples. Positive detection of the resistance genes in the recent years highlights the significant health threaten phenomena and highly recommends the wise use of antimicrobials and even replace its application with trends of biosecurity and probiotics.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; BUFVTM21/11/2023).

TABLE 1. Oligonucleotide primers sequences

Bacteria	Gene	Sequence	Amplified product	Reference
Staphylococcus	mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure et al.[33]
	vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	Patel et al. [34]
	tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	Duran et al.[35]
E. coli	blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom et al., [36]
	TetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	570 bp	Randall et al. [37]
	Aada1	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484 bp	Randall et al. [37]

DNA Extraction was performed following QIAamp DNA mini kit instructions (cat.no. 51304).
Amplification program of the examined genes was adjusted according to the kit instruction.
Agarose gel electrophoreses was performed according to Sambrook et al. (1989).
2.4. Statistical analysis

TABLE 2. Incidence of *E. coli* and *S. aureus* in the examined meat samples (n=50).

Samples	*E. coli*		*S. aureus*	
	No.	%	No.	%
Chevon meat	18	36*	22	44*
Mutton meat	25	50*	26	52*
Total	**43**	**43****	**48**	**48****

* Incidence in relation to the number of each sample (n=50)
** Incidence in relation to the total number of the examined samples (n=100)

TABLE 3. In-Vitro anti-microbial Sensitivity test for isolated *E. coli* strains of the examined mutton meat samples (n=25).

Antimicrobial agents			Sensitive		Resistant		AA
Agent	Symbol	Conc. (µ/g)	No.	%	No.	%	
Amoxicillin	AX	10 µg	8	32.0	17	68.0	R
Ampicillin / salbuctam	SAM	20 µg	10	40.0	15	60.0	R
Azithromycin	AZM	15 µg	6	24.0	19	76.0	R
Cefaclor	CEC	30 µg	5	20.0	20	80.0	R
Cefoxitin	FOX	30 µg	19	76.0	6	24.0	S
Ceftriaxone	CRO	30 µg	11	44.0	14	56.0	R
Colistin	CT	10 µg	9	36.0	17	64.0	R
Doxycycline	DO	30 µg	7	28.0	18	72.0	R
Norfloxacin	NR	10 µg	20	80.0	5	20.0	S
Oxacillin	OX	5 µg	10	40.0	15	60.0	R
Tetracycline	TE	10 µg	5	20.0	20	80.0	R

TABLE 4. In-Vitro anti-microbial Sensitivity test for isolated *E. coli* strains of the examined chevon meat samples (n=18).

Antimicrobial agents			Sensitive		Resistant		AA
Agent	Symbol	Conc. (µ/g)	No.	%	No.	%	
Amoxicillin	AX	10 µg	5	27.8	13	72.2	R
Ampicillin / salbuctam	SAM	20 µg	5	27.8	13	72.2	R
Azithromycin	AZM	15 µg	12	66.7	6	33.3	S
Cefaclor	CEC	30 µg	3	16.7	15	83.3	R
Cefoxitin	FOX	30 µg	12	66.7	6	33.3	S
Ceftriaxone	CRO	30 µg	8	44.4	10	55.6	R
Colistin	CT	10 µg	6	33.3	12	66.7	S
Doxycycline	DO	30 µg	5	27.8	13	72.2	R
Norfloxacin	NR	10 µg	11	61.1	7	38.9	S
Oxacillin	OX	5 µg	17	94.4	1	5.6	S
Tetracycline	TE	10 µg	3	16.7	15	83.3	R

Furthermore, the isolated *S. aureus* strains showed lower resistance levels than *E. coli* isolates against the used antibiotics; where they were almost sensitive to azithromycin, cefaclor, cefoxitin, ceftriaxone and norfloxacin **(Tables 5 and 6).**

TABLE 5. In-Vitro anti-microbial Sensitivity test for isolated *S. aureus* strains of the examined mutton meat samples (n=26).

Antimicrobial agents			Sensitive		Resistant		AA
Agent	**Symbol**	**Conc. (µ/g)**	**No.**	**%**	**No.**	**%**	
Amoxicillin	AX	10 µg	5	19.2	21	80.8	R
Ampicillin / salbuctam	SAM	20 µg	8	30.8	18	69.2	R
Azithromycin	AZM	15 µg	20	76.9	6	23.1	S
Cefaclor	CEC	30 µg	18	69.2	8	30.8	S
Cefoxitin	FOX	30 µg	15	57.7	11	42.3	S
Ceftazidime	CAZ	10 µg	12	46.2	14	53.8	R
Ceftriaxone	CRO	30 µg	19	73.1	7	26.9	S
Doxycycline	DO	30 µg	9	34.6	17	65.4	R
Norfloxacin	NR	10 µg	18	69.2	8	30.8	S
Oxacillin	OX	5 µg	5	19.2	21	80.8	R
Streptomycin	S	10 µg	17	65.4	9	34.6	R
Tetracycline	TE	10 µg	22	84.6	4	15.4	R

TABLE 6. In-Vitro anti-microbial Sensitivity test for isolated *S. aureus* strains of the examined chevon meat samples (n=22).

Antimicrobial agents			Sensitive		Resistant		AA
Agent	**Symbol**	**Conc. (µ/g)**	**No.**	**%**	**No.**	**%**	
Amoxicillin	AX	10 µg	3	13.6	19	86.4	R
Ampicillin / salbuctam	SAM	20 µg	8	36.4	14	63.6	R
Azithromycin	AZM	15 µg	18	81.8	4	18.2	S
Cefaclor	CEC	30 µg	15	68.2	7	31.8	S
Cefoxitin	FOX	30 µg	17	77.3	5	22.7	S
Ceftazidime	CAZ	10 µg	4	18.2	18	81.8	R
Ceftriaxone	CRO	30 µg	19	86.4	3	13.6	S
Doxycycline	DO	30 µg	16	72.7	6	27.3	S
Norfloxacin	NR	10 µg	18	81.8	4	18.2	S
Oxacillin	OX	5 µg	20	90.9	2	9.1	S
Streptomycin	S	10 µg	5	22.7	17	77.3	R
Tetracycline	TE	10 µg	8	36.4	14	63.6	R

Referring to the obtained results of the molecular identification of drug resistance genes of the examined *E. coli* isolates, *tetA* and *blaTEM* were detected in 100% of the examined isolates **(Fig. 1)**; whereas, aada1 gene were detected in 80% of the examined isolates **(Fig. 2)**. On the other hand, *mecA* and *tetk* were detected in 80% of the examined *S. aureus* isolates **(Fig. 3)**, while *vanA* gene was detected in only 40% of the examined samples **(Fig. 4).**

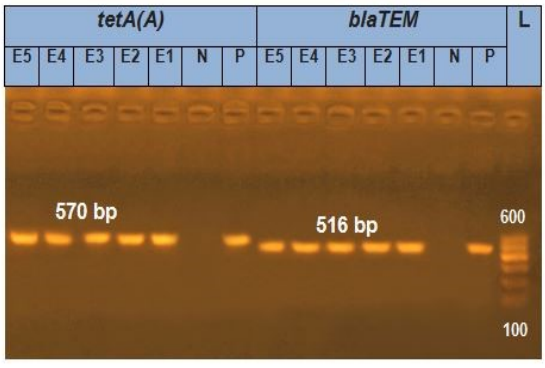

Fig. 1. Agarose gel electrophoresis of *blaTEM* (516 bp) and *tetA*(A) (570 bp) for *E. coli*.
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive for *blaTEM* and *tetA* genes.
Lane N: Control negative.
Lanes E1-E5: Positive *E. coli* strains for the examined genes

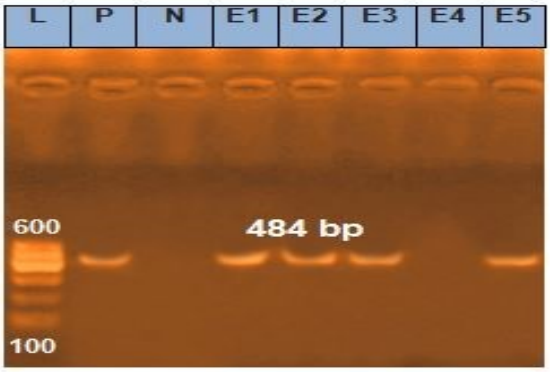

Fig. 2. Agarose gel electrophoresis of *aada1* (484 bp) for *E. coli*.
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive for *aada1* gene.
Lane N: Control negative.
Lanes E1, 2, 3, 5: Positive *E. coli* strains for *aada1* gene
Lane 4: Negative *E. coli* strain for *aada1* gene.

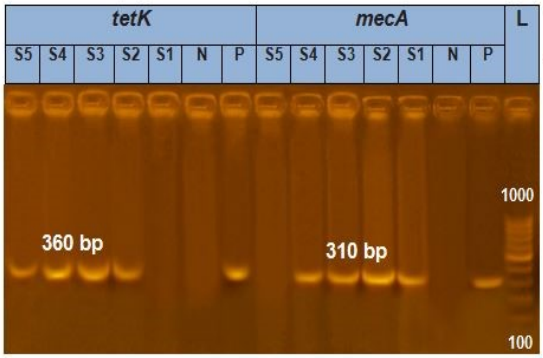

Fig. 3. Agarose gel electrophoresis of *mecA* (310 bp) and *tetK* (360 bp) for *S. aureus*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive for *mecA* and *tetK* genes.
Lane N: Control negative.
Lanes S1-S4: Positive *S. aureus* strains for *mecA* gene
Lane S5: Negative *S. aureus* strains for *mecA* gene
Lanes S2-S4: Positive *S. aureus* strains for *tetK* gene
Lane S1: Negative *S. aureus* strains for *tetK* gene

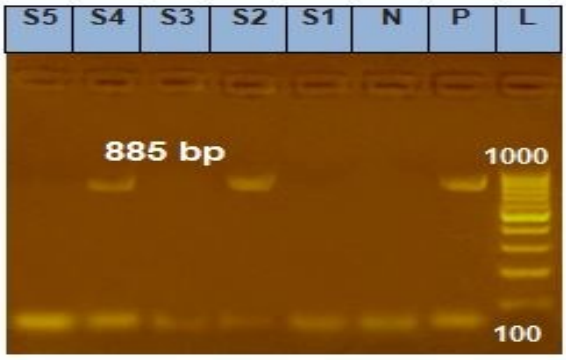

Fig. 4. Agarose gel electrophoresis of *vanA* (885 bp) for *S. aureus*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive for *vanA* gene.
Lane N: Control negative.
Lanes S2 & S4: Positive *S. aureus* strain for *vanA* gene
Lanes S1, S3 & S5: Negative *S. aureus* strain for *vanA* gene.

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دراسة ميكروبيولوجية تفاضلية على البكتيريا المقاومة للمضادات الحيوية من لحم الماعزوالضأن الخام

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الملخص

تم تصميم الدراسة الحالية لمعرفة مقاومة مضادات الميكروبات التي أصبحت قضية عالمية رئيسية للإنسان والحيوان. أصبح الاستخدام المتزايد للمضادات الحيوية في الماشية أحد أسباب تطور مقاومة مضادات الميكروبات في البكتيريا المنقولة بالغذاء. كان الهدف من الدراسة الحالية هو تحديد خصائص البكتيريا المقاومة للمضادات الحيوية، الإشريكية القولونية والمكورات العنقودية الذهبية، من مائة لحم غنم نيئ ولحوم ضأن (50 من كل منهما) تم جمعها من مسالخ عشوائية في محافظة القليوبية بتقنيات قياسية في المختبر وفقًا لإرشادات معهد المعايير السريرية والمعملية (CLSI). من بين 100 عينة، تم الكشف عن الإشريكية القولونية في 36٪ و 50٪، بينما تم الكشف عن المكورات العنقودية الذهبية في 44٪ و 52٪ من لحوم غنم ولحم ضأن تم فحصها، مما يكشف عن أن لحم الضأن أكثر تعرضًا للتلوث البكتيري من غنم. أظهرت نتائج المضادات الحيوية المختلفة التي تحد من العزلات المكتشفة مستويات عالية من المقاومة ضد المضادات الحيوية المختلفة المستخدمة؛ حيث أظهرت عزلات لحم الضأن مستويات مقاومة أعلى من عزلات شيفون. تم الإبلاغ عن أعلى نسب مقاومة ضد التتراسيكلين والسيفاكلور والأموكسيسيلين. في حين كانت حساسة تقريبًا للأزيثروميسين والسيفترياكسون والنورفلوكساسين. بالإشارة إلى التعريف الجزيئي لجينات مقاومة الأدوية، تم الكشف عن tetA و blaTEM في جميع عزلات E. coli المدروسة؛ بينما تم الكشف عن جين aada1 في 80٪ من العزلات المدروسة. من ناحية أخرى، تم الكشف عن mecA و tetk في 80٪ من عزلات S. aureus المدروسة، بينما تم الكشف عن جين vanA في 40٪ فقط من العينات المدروسة. لذلك، يوصى بشدة بظروف صحية صارمة والاستخدام الحكيم للمضادات الحيوية في الماشية.

الكلمات الدالة: اللحوم الطازجة ، مقاومة الأدوية المتعدد ، المسالخ.