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Original Article
Özgün Araştırma

Phenotypic and genotypic investigation of antibiotic resistance properties from different animals feces

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Abstract: In this study, antibiotic resistance, Extended-Spectrum Beta-Lactamase (ESβL), Ampicillin-C (Amp-C), and Carbapenemase activities were investigated in *Escherichia coli* (E. coli) isolates obtained from sheep, goat, and cattle feces in Kırıkkale Province. The samples were identified using conventional methods and rapid diagnostic kits, and their resistance profiles were evaluated using the Kirby-Bauer disk diffusion method. Phenotypic tests, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), were used to determine ESβL, Amp-C, and Carbapenemase activities. Positive samples were further analyzed genotypically using PCR to detect resistance genes. Resistance was most commonly observed in sheep isolates, followed by cattle and goats, particularly against trimethoprim/sulfamethoxazole and gentamicin. Phenotypic tests revealed 22 (24.4%) ESβL-positive isolates and one suspected Carbapenemase producer, while no Amp-C activity was detected. Genotypic analysis identified ESβL genes in 4 cattle (13.3%), 7 sheep (23.3%), and 11 goat (36.6%) isolates, with the SHV gene being the most prevalent. The single isolate phenotypically suspected of carbapenemase activity tested negative by PCR. Although the absence of Amp-C and carbapenemase activity is reassuring, the significant prevalence of ESβL-producing isolates raises concerns about the spread of antibiotic resistance. This highlights the need for monitoring and prudent antibiotic use in livestock management.

Keywords: Amp-C, Antimicrobial resistance, Carbapenemase, *E. coli,* ESβL, Feces.

Farklı hayvan dışkılarından antibiyotik direnç özelliklerinin fenotipik ve genotipik incelenmesi

Özet: Kırıkkale İli ve çevresindeki koyun, keçi ve sığır dışkılarından izole edilen *Escherichia coli* (*E. coli*) izolatlarının antibiyotik dirençliliği, geniş spektrumlu Beta-laktamaz (GSβL), Ampicilin-C (Amp-C) ve Karbapenemaz aktiviteleri fenotipik ve genotipik olarak incelenmiştir. Toplanan 30 sığır, 30 koyun ve 30 keçi izolatında, Kirby-Bauer disk difüzyon yöntemi ile çoklu ilaç direnci araştırılmıştır. Fenotipik olarak GSβL, Amp-C ve Karbapenemaz aktiviteleri Klinik Laboratuvar Standartları Enstitüsü (CLSI) doğrulama testleriyle değerlendirilmiş; GSβL genleri (CTX-M1, OXA-1, CTX-M9, SHV, TEM), Amp-C geni (FOX) ve Karbapenemaz genleri (IMP, KPC, OXA-48, VIM, NDM) klasik PCR ile incelenmiştir. Testler sonucunda direnç sıklığının sırasıyla koyun, sığır ve keçi izolatlarında en yüksek olduğu; en yüksek direnç oranının ise trimethoprim/sulfametoksazol ve gentamisin antibiyotiklerine karşı geliştiği gözlenmiştir. Fenotipik olarak 22 (%24,4) GSβL pozitif, 1 (%1,1) karbapenemaz şüpheli izolat belirlenmiş; Amp-C pozitiflik gözlenmemiştir. Genotipik incelemede GSβL üretimi 4 sığır (%13,3), 7 koyun (%23,3) ve 11 keçi (%36,6) izolatında tespit edilmiş, SHV tipi genin yaygın olduğu belirlenmiştir. Karbapenemaz şüpheli sığır izolatı PCR ile negatif bulunmuştur. Sonuçlar, Amp-C ve karbapenemaz aktivitesinin saptanmamasının olumlu olduğunu; ancak yüksek GSβL üretiminin antibiyotik dirençliliğinin yayılımı açısından önemli bir sorun teşkil ettiğini göstermektedir.

Anahtar kelimeler: Amp-C, Antimikrobiyal direnç, *E. coli,* Gaita, GSβL, Karbapenemaz

Introduction

Resistance to beta-lactamase group antibiotics, which are widely used in the treatment of bacterial infections in humans and animals, is a global problem. These resistance mechanisms are mainly distinguished in *Enterobacteriacea* family which is includes *Escherichia coli* (*E. coli),* but it also can seen in many bacteria (Güven and Kızıl 2022; Mahmood and Ahmed 2022). Bacteria with antimicrobial resistance continue to spread the resistance easily and rapidly throughout the world, with various mechanisms such as food chain and cross-contamination. *E. coli* strains showing Extended Spectrum Beta-lactamase (ESβL), Ampicillin-C **(**Amp-C) and Carbapenemase activity bring many problems in terms of animal health and public health (Güven and Kızıl 2022).

ESβL genes secreting by plasmids and transposons, which causes to resistant bacterias to penicillins, cephalosporins, monobactams and can be easily transferred genetically (Shabana and Al-Enazi

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2020). Beta-lactamases are classified according to their substrate-inhibitory structure and functions. Amp-C gene, which is in group C in Ambler classification, is a cephalosporinase encoded in chromosomes. It has been reported, that Amp-C genes has plasmid origins, frequently co-occur with ESβL genes (Silva et al. 2020).

Carbapenems, which are the broadest spectrum beta-lactam group antibiotics, are used against ESβL and Amp-C producing bacteria. In recent years, the development of resistance to these antibiotics has been observed (Dallane et al. 2010; Mahmood and Ahmed 2022). Carbapenemase that hydrolyzes carbapenem enzymes show activity against both this group of antibiotics and beta-lactam group antibiotics. The resulting resistance mechanisms lead to the development of multi-drug resistance, adversely affecting the duration and outcome of treatment, and increase the mortality rate (Pehlivanoğlu 2019).

In this study, *E. coli* strains that isolated from cattle, sheep and goat feces were investigated phenotypically to see ESβL, Amp-C, Carbapenemase activities and multi-drug resistance (MDR). The presence of antibiotic resistance genes in resistant strains was investigated by PCR.

Material and Method

Isolation and Identification: Fecal samples collected from healthy cattle, sheep and goats around Kırıkkale province with sterile swab and were brought to the laboratory in a cold chain as soon as possible. Inoculation was made on Blood Agar, Nutrient Agar and MacConkey and incubated at 37°C for 18-24 hours. *E. coli* identification was made by using conventional methods and rapid diagnosis kit (BBL- Crystal) (Aslantaş et al. 2017).

Determination of Antibiotic Susceptibilities of Isolates: Identified E. *coli* isolates were evaluated in terms of resistance profile and multidrug resistance by Kirby-Bauer disk diffusion method and cefotaxime, aztreonam, ceftazidime, amoxicillin/clavulanic acid, amikacin, trimethoprim/sulfamethoxazole, gentamicin, imipenem, tobramycin, ceftriaxone, ciprofloxacin, cefepime, ertapenem, cefoxitin disks were examined. Test results evaluated by using EUCAST and CLSI database for *Enterobacterales* and *E. coli ATCC* 25922 strain was used as a positive control in the study. As a result of the disk diffusion test, the strains were also examined for MDR (EUCAST 2022).

As a result of disk diffusion test ceftazidime, and cefotaxime antibiotics were evaluated according to ESβL screening test criteria. The samples with positive screening test results searched by looking at the zone difference between the combinations cefotaxime and cefotaxime/clavulanic acid and/or ceftazidime and ceftazidime/clavulanic, samples with a diameter of 5 mm or more were confirmed for ESβL activity.

In addition, the resistance development of isolates against cefoxitin was examined and evaluated in terms of Amp-C activity. As the carbapenemase screening test, the ertapenem zone diameters were checked. The isolates identified as suspicious, were subjected to a confirmatory test with the modified hodge test. For this test, 0.5 McFarland *E. coli* ATCC 25922 strain was diluted 1:10 and then inoculated into Muller-Hinton medium and an ertapenem disk was placed in the center. 3-5 colonies were taken from the suspicious sample and plated in a straight line on the disk edge. While *E. coli* ATCC 25922 strain was used as negative control, CLSI (2018) criteria were used for evaluation.

Detection of ESBL, Amp-C and Carbapenemase Resistance by PCR: DNA isolation from the *E. coli* samples done by boiling method. For this purpose, one colony was taken from each of the isolates and suspended with 100 microliter (µl) sterile distilled water. The bacterial suspension prepared in Eppendorf tubes was boiled at 95 °C for 10 minutes and then centrifuged at 10,000 rpm for 2 minutes. The supernatant part of the suspension was taken into sterile tubes and the amount of DNA was determined in ng/mL by nanodrop spectrophotometer (Dallenne et al. 2010).

CTXM-9, CTXM-1, SHV, TEM and OXA-1 genes for ESBL activity; FOX gene for Amp-C activity and OXA-48, IMP, VIM, KPC and NDM-1 genes were used to determine carbapenemase activity (Dallenne et al. 2010).

For conventional PCR 14µl sterile distilled water, 4 µl master mix, 1 µl DNA, 0.5 µl forward primer, 0.5 µl reverse primer prepared total of 20 µl. PCR protocol applied as 10 minutes at 94°C, 40°C at 94°C 30 cycles per second, 40 seconds at 60°, 1 minute at 72°, 7 minutes at 70°.

Thereafter PCR amplification, DNAs executed in 1% agarose gel at 100 volts for 30 minutes and under UV light gene activities in gel examined.

Kappa test was used in the compatibility comparison of the tests that performed and the results were evaluated based on M. Hugh (2012) values.

Results

When the highest resistance development to antibiotics examined among animal species; results respectively detected as sheep, cattle and in goats. Highest antibiotic resistance was determined trimethoprim/sulfamethoxazole 13.33%, gentamycin 8.88% and amoxicillin/clavulanic acid 6.66%. As a result of disk diffusion tests, resistance in other antibiotics percentage found as ciprofloxacin 5.55%, ceftriaxone 4.44%, amikacin 3.33%, aztreonam 1.11%, cefotaxime 1.11%, tobramycin and ertapenem 1.11%. The development of resistance to the antibiotics used was found as 46.66% (42) of the ninety *E. coli* isolates, and multi-drug resistance was found as 10% (9) (Table 1).

Table 1. Disk Diffusion Test Results

Antibiotics	S	I	R
Amikacin	96.7% (87)	0% (0)	3.3% (3)
Amoxicillin/ clavulanic acid	93.4% (84)	0% (0)	6.6% (6)
Aztreonam	96.7% (87)	2.2% (2)	1.1% (1)
Cefepime	98.9% (89)	0% (0)	1.1% (1)
Cefotaxime	97.8% (88)	1.1% (1)	1.1% (1)
Cefoxitin	100% (90)	0% (0)	0% (0)
Ceftazidime	96.7% (87)	3.3% (3)	0% (0)
Ceftriaxone	92.3% (83)	3.3% (3)	4.4% (4)
Ciprofloxacin	90% (81)	4.4% (4)	5.5% (5)
Ertapenem	98.9% (89)	0% (0)	1.1% (1)
Gentamicin	91.2% (82)	0% (0)	8.8% (8)
Imipenem	100% (90)	0% (0)	0% (0)
Tobramycin	98.9% (89)	0% (0)	1.1% (1)
Trimethoprim/ sulfamethoxazole	85.5% (77)	1.1% (1)	13.4% (12)

As a result of the ESβL screening test, 22 (24.4%) samples were considered suspicious. ESβL activity was phenotypically positive in 21 (23.3%) of the samples subjected to the confirmatory test with the combined disk diffusion test. When animal species were compared, ESβL activity was found to be 47.6% (10) in goats, 33.4% (7) in sheep, and 19(4)% in cattle (Table 2).

As a result of examining the development of resistance to cefoxitin, which is used as a screening test for Amp-C activity, no positivity was detected in any of the samples. When the isolates were examined in terms of carbapenemase activity, resistance to ertapenem was detected in only 1 of 90 isolates in the screening test. As a result of the confirmation test, the carbapenemase activity of this isolate was negative (Table 2).

Table 2. Phenotypic Test Results for ESβL, Amp-C and Carbapenemase Activity

Animals	Isolates number (%)		
	ESβL	Amp-C	Carbapenemase
Cattle	4 (19%)	0 (0%)	0 (0%)
Sheep	7 (33.4%)	0 (0%)	0 (0%)
Goat	10 (47.6%)	0 (0%)	0 (0%)

When 22 suspicious samples with positive ESβL screening test results were examined by PCR, positive genes were detected in all samples. While SHV gene was observed in all isolates examined, the presence of CTX-M1 detected as 86.4%, CTX-M9 68.2%, TEM 63.6%, OXA-1 gene 36.4%. It was determined that the highest resistance genes in all animal species were SHV and CTX-M1. While all resistance genes were detected in 13.6% of the isolates examined by PCR, 4 resistance genes were detected in 40.90%. OXA-1 and SHV genes were determined as positive in a goat isolate that was negative as a result of the ESβL confirmation test (Table 3).

As a result of the carbapenemase screening test, isolates that showed ertapenem resistance is examined in terms of gene activity by PCR; OXA-48, IMP, VIM, KPC and NDM-1 genes were not detected (Table 3).

Table 3. PCR Results of ESβL, Amp-C and Carbapenemase Activity

Animals	Isolates number (%)		
	ESβL	Amp-C	Carbapenemase
Cattle	4 (18,2%)	0 (0%)	0 (0%)
Sheep	7 (31.8%)	0 (0%)	0 (0%)
Goat	11 (50%)	0 (0%)	0 (0%)

Comparison of phenotypic and genotyping results is given in Table 4.

Table 4. Phenotypic and Genotypic Comparison

Animals	Phenotypic isolates number (%)			Genotypic isolates number (%)		
	ESβL	Amp -C	Carbapenemase	ESBL	Amp -C	Carbapenemase
Cattle	4 (19%)	0 (0%)	0 (0%)	4(18.2%)	0 (0%)	0 (0%)
Sheep	7(33.4%)	0 (0%)	0 (0%)	7(31.8%)	0 (0%)	0 (0%)
Goat	10 (47.6%)	0 (0%)	0 (0%)	11 (50%)	0 (0%)	0 (0%)

Almost perfect agreement was found between ESβL screening-confirmation (p= 0.92) and Carbapenemase screening-validation (p=1) tests. When the agreement between ESβL validation and PCR test was examined, almost perfect agreement was found, while looking at the gene level, a strong agreement was determined between the combined disk and PCR (OXA-1). The results are given in Table 5.

Table 5. Statistics (Kappa Test) Results

TESTS	P	Evaluation
Disk Diffusion- Combined Disk Diffusion	0.92	Almost Perfect Fit
Disk Diffusion- Modified Hodge	1.00	Almost Perfect Fit
Combined Disk- PCR	1.00	Almost Perfect Fit
Combined Disk- PCR (CTXM-1)	1.06	Almost Perfect Fit
Combined Disk- PCR (OXA-1)	0.86	Strong Compliance
Combined Disk- PCR (CTXM-9)	1.15	Almost Perfect Fit
Combined Disk- PCR (SHV)	1.00	Almost Perfect Fit
Combined Disk- PCR (TEM)	1.06	Almost Perfect Fit

Discussion and Conclusion

E. coli, which is one of the most important bacteria found in the intestinal flora of animals and humans also can found widely in plants, soil and water; is one of the head reason of health problems affecting the whole world with the development of antimicrobial resistance (Silva et al. 2020). Studies on the development of agents and resistance are carried out in many countries.

Similar to our study in Turkey, *E. coli* strains isolated from faeces swabs and isolates were examined in terms of antimicrobial resistance and resistance genes. In 2016, high resistance to trimethoprim/sulfamethoxazole (76.9%) and amoxicillin/clavulanic acid (46.2%) were found in *E. coli* samples isolated from cattle faeces swabs in the Hatay region, while MDR was detected at a rate of 84.6%. In addition, ESβL positivity was detected in 26 of these isolates as a result of phenotypic tests, and multiple resistance phenotypes were detected in 22 of these samples in PCR studies. The most common resistance genes were determined as TEM and CTX-M1 (Aslantaş et al. 2017).

E. coli isolated from calf fecal swabs in 2018 and from cattle and sheep faeces swabs in 2020 in Burdur region. Similarly, in the samples of trimethoprim/sulfamethoxazole, gentamicin resistance was detected to a high degree (14, 23). While ESβL positivity was detected in 24% of the calf isolates, the CTX-M (100%) and TEM (91.66%) genes were reported at high rates. In the study conducted in 2020, 51 cattle *E. coli* ESβL positivity was detected in 17 of the isolates. In all 34 ESβL negative isolates, resistance to cefoxitin was determined as a result of disk diffusion test and it was stated that potential Amp-C activity might be present (Pehlivanoğlu et al. 2020). When these studies conducted in recent years and our results are compared, it is noteworthy that the SHV gene we obtained because of PCR was obtained from all isolates (Aslantaş et al. 2017; Yıldırım and Pehlivanoğlu 2018; Pehlivanoğlu et al. 2020).

In 2018, Rawat et al. found ceftazidime and amoxicillin/clavulanic resistance in 66 *E. coli* isolates identified from cattle feces in India. Acid antibiotics were found to be highly resistant, while 36 samples were positive as a result of the ESβL confirmation

test. The most common SHV (36.6%) and TEM (30.3%) genes were reported in isolates. Similar to our study, KPC and NDM genes were not found in isolates (Rawat et al. 2018).

In a study conducted in 2020 in Saudi Arabia, a total of 157 sheep and 77 goat fecal samples were collected from both healthy and diseased animals. Among these samples, *E. coli* was isolated from 197 of them. High resistance to the antibiotic's ciprofloxacin, kanamycin, and gentamicin was detected in these isolates. PCR results revealed a significant presence of the CTX-M9 and CTX-M1 genes, with a higher occurrence observed in the fecal samples from diseased animals (Shabana and Al-Enazi 2020).

In another study conducted in Spain in 2020, ESβL activity was found to be 24% in dairy cattle, 8.74% in beef cattle and 6.1% in sheep; Amp-C activity was determined as 6.1% in dairy cattle, 1% in beef cattle and 0.9% in sheep. As a result of the MIC test performed in the study, the presence of resistance against cefepim, ceftazidime, ciprofloxacin and gentamicin antibiotics was detected; No resistance to imipenem, meropenem and colistin was found. When the whole genome was sequenced, the most common resistance gene was found to be CTX-M with a rate of 90.9%, while the SHV gene was found sporadically (Tello et al. 2020).

Results of study in Iraq in 2022, a total of 40 *E. coli* isolates were collected from milk and fecal samples of healthy sheep, lambs, and sheep with clinical mastitis. These isolates were then subjected to genotypic analysis using PCR. Among the 40 isolates, 29 were derived from fecal samples. When focusing on the isolates obtained from fecal samples in this study, the most prevalent genes were CTX-M (100%), followed by SHV (24.14%), and TEM (13.8%) genes. No *E. coli* isolate carrying the Amp-C resistance gene was detected in the study (Mahmood and Ahmed 2022).

When we look at the studies conducted in Turkey and around the world in recent years, the high amount of *E. coli* isolation from cattle, sheep and goat feces, the high antimicrobial resistance in these isolates, and the increase in ESβL activity have drawn attention. Our study is the first time that the phenotypic and genotypic investigation of antimicrobial resistance in isolates has been carried out in Kırıkkale Province. In addition, our study showed that the SHV resistance gene increased in *E. coli* isolates showing ESβL activity, while the CTX-M gene was also determined at a high rate. Although the absence of Amp-C and carbapenemase activity is reassuring, the significant prevalence of ESβL-producing isolates raises concerns about the spread of antibiotic resistance. This highlights the need for monitoring and prudent antibiotic use in livestock management.

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References

Abdelmoktader A, Far ATE (2019): Methods of ESβLs detection in clinical microbiology lab. *Virol Immunol J*. 3, 000222. DOI: 10.23880/vij-16000222

Akçam FZ, Gönen İ, Kaya O et al. (2004): Hastane infeksiyonu etkeni Enterobakterilerde Beta-laktam antibiyotiklere duyarlılık ve ESBL sıklığının araştırılması. *Med J SDU.* 11, 6-9.

Aslantaş Ö, Elmacıoğlu S, Yılmaz EŞ (2017): Prevalence and characterization of ESβL- and AmpC-producing *Escherichia coli* from cattle. *Kafkas Univ Vet Fak Derg*. 23, 63-67. DOI: 10.9775/kvfd.2016.15832

Biz E, Kutlu HH, Tekeli A (2016): Comparison of modified hodge test and inhibitor based tests for detection of Carbapenemase producer *Enterobacteriaceae* isolates. *J Ankara Üniv Fac Med.* 69, 151-157. DOI: 10.1501/Tipfak_0000000937

Clinical and Laboratory Standards Institute (2018) *The modified hodge test for suspected Carbapenemase production in Enterobacteriaceae.* 28th Edition, 2-4. Wayne, PA, USA.

Dağlar D, Öngüt G (2012): Extended Spectrum Beta-Lactamases (ESβL) and diagnostic methods. *JHVS.* 1, 1-9

Dallenne C, Costa A, Decre D et al. (2010): Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae. J AntimicroChemother.* 490-495. DOI: 10.1093/jac/dkp498

Egbule OS, Yusuf İ (2019*):* Multiple Antibiotics Resistance s in *Escherichia coli* isolated from Cattle and Poultry Faeces in Abraka- South Nigeria. *Pertanika J Trop Agric Sci*. 42, 585-594.

Gülay Z (2002*):* Interpretation of Antibiotic Susceptibility Tests. *Türk Thorax Derg.* 3,75-88.

Güven G, Kızıl S (2022*):* Investigation of antimicrobial resistance of ESβL, Amp -C, and carbapenemase producing *E. coli* strains in retail poultry meat. *Turk J Vet Anim Sci.* 46, 788-794. DOI: 10.55730/1300-0128.4254

Mahmood FR, Ahmed IM (2022): Molecular detection of ESβL, Amp-C, ß- Lactamase *Escherichia coli* isolated from sheep in Mosul city. *Iraqi J Vet Sci*. 36, 387-392. DOI: 10.33899/ijvs.2021.130380.1810

Mc Hugh, M.L. (2012): Interrater reliability: the kappa statistic. Biochemia Medica 22 (3): 276-282.

Oberle K, Capdeville MJ, Berthe T et al. (2012): Evidence for a complex relationship between antibiotics and antibiotic resistant *Escherichia coli* from medical patients to a receiving environment. *Environ Sci Technol.* 46, 1859-1868. DOI: 10.1021/es203399h

Ögedey E, Cömert F, Köktürk F et al. (2016): Extended Spectrum Beta-Lactamase (ESβL) producing *Escherichia coli* and *Klebsiella spp.* determination of *CTX-M* enzymes in isolates. *Turkish Society Microbiol*. 46, 88-96. DOI: 10.5222/TMCD.2016.088

Öztürk CE, Kaya AD, Yücel M et al. (2010): Comparison of some phenotypic tests used in Extended Spectrum Beta-Lactamase detection. *ANKEM*. 24, 111-116.

Pehlivanoğlu F, Özturk D, Turutoğlu H (2020): Carriage of plasmidic AmpC, Beta-Lactamase producing *Escherichia coli* in cattle and sheep and characterization of the isolates in terms of antibiogram profiles, phylogeny and virulence. *Kafkas Univ Vet Fak Derg*. 26, 469-476. DOI: 10.9775/kvfd.2019.23541

Pehlivanoğlu F (2019): Beta-Lactamase enzyme diversity of Gram-negative bacteria and the situation in bacteria of anamal origin in Turkey. *F U Vet J Health Sci*. 33, 113-122.

Plassard V, Gisbert P, Granier S et al. (2021): Surveillance of Extended Spectrum β-Lactamase, Cephalosporinase and Carbapenemase Producing Gram-negative bacteria in raw milk filters and healthy dairy Cattle in Tree Farms in Ile-de-France, France. *Front Vet Sci*. 8, 633598. DOI: 10.3389/fvets.2021.633598

Rawat N, Singh F, Hirpurkar SD et al. (2018): Detection and characterization of Extended-Spectrum Beta-Lactamase Genes *(bla TEM and bla SHV)* among Beta-Lactam resistant fecal coliforms of dairy cattle from Chhattisgarh, India. *Turk J Vet Anim Sci*. 42, 503-511. DOI: 10.3906/vet-1706-77

Shabana II, Al- Enazi AT (2020): Investigation of plasmid-mediated resistance in *E. coli* isolated from healthy and diarrheic sheep and goats. *Saudi J Biol Sci*. 27, 788-796. DOI: 10.1016/j.sjbs.2020.01.009

Silva N, Carvalho I, Currie C et al. (2020): *Extended-Spectrum β-Lactamase and Carbapenemase-Producing Enterobacteriaceae in food-producing animals in Europe*. 261-273, Antibiotic Drug Resistance First Edition. DOI: 10.1002/9781119282549.ch12

Tello M, Ocejo M, Oporto B et al. (2020): Prevalence of Cefotaxime resistant *Escherichia coli* isolates from healthy cattle and sheep in Northern Spain: phenotypic and genome-based characterization of antimicrobial susceptibility. *Appl Environ Microbiol*. 86, e00742-20. DOI: 10.1128/AEM.00742-20

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2022): Breakpoint tables for interpretation of MICs and zone diameters. Version 13.0. Access address: https://www.eucast.org/clinical_breakpoints, Access date: 04.02.2025.

Yıldırım D, Pehlivanoğlu F (2018): Extended Spectrum Beta Lactamase producing *Escherichia coli* in calves. *F U Vet J Health Sci*. 32, 155-160.