Polish Journal of Veterinary Sciences Vol. 27, No. 4 (2024), 537–546

DOI 10.24425/pjvs.2024.151749

Original article

Carriage of antimicrobial resistance genes in *Escherichia coli* of bovine origin

G. Ozbey[1], E.S. Tanriverdi[2], M.N. Acik[3], R. Kalin[4], B. Otlu[2], F. Zigo[5]

[1] Department of Medical Services and Techniques, Vocational School of Health Services, Firat University, TR-23119 Elazig, Turkey
[2] Department of Medical Microbiology, Faculty of Medicine, Inonu University, TR-44280 Battalgazi, Malatya, Turkey
[3] Department of Microbiology, Faculty of Veterinary Medicine, Bingol University, TR-12000 Bingol, Turkey
[4] Department of Microbiology, Faculty of Veterinary Medicine, Cumhuriyet University, TR-58140 Sivas, Turkey
[5] Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, Košice, 04181, Slovakia

Correspondence to: F. Zigo, tel.: +421 908 689 722, e-mail: frantisek.zigo@uvlf.sk

Abstract

The present study aimed to search for the presence of the plasmid-mediated antimicrobial resistance genes in 106 *Escherichia coli* (*E. coli*) isolates from a total of 240 fresh fecal samples collected from 12 private cattle farms in Bingol province of East Turkey from November 2021 to January 2022. In those colistin-resistant *E. coli* (*mcr*-1 to -9), the major carbapenemase (bla_{OXA-48}, bla_{NDM-1}, $bla_{IMP,\ bla VIM,}$ and bla_{KPC}), β-lactamase (bla_{TEM-1}, bla_{CTX-M} and bla_{SHV-1}) and OXA-48 like β- lactamase ($bla_{OXA-162}$, $bla_{OXA-163}$, $bla_{OXA-181}$, $bla_{OXA-204}$ and $bla_{OXA-232}$) resistance genes were searched for determined a multiplex polymerase chain reaction (PCR) method and Next-generation sequencing (NGS) - PCR Amplicons with Nanopore Technology. Only the *mcr*-4 gene was found in one isolate and the remaining genes (*mcr*-1-9) were not shown in all *E. coli* isolates from cattle. The minimal inhibitory concentration (MIC) to colistin was detected in *mcr*-4 positive *E. coli* isolates using broth microdilution. We assessed the antimicrobial susceptibilities of *mcr*-4 positive *E. coli* isolates using the Kirby-Bauer disk diffusion method. *E coli* isolate was detected as negative for carbapenemase and OXA-48 like β-lactamase resistance genes and positive for β-lactamase. In addition, *E. coli* isolates carrying *mcr*-4 were more resistant to colistin. Antimicrobial susceptibility testing using the disk diffusion assay indicated that all 106 *E. coli* isolates (100%) were sensitive to AMK, 105 *E. coli* isolates (99.1%) exhibited sensitivity to imipenem, meropenem and doripenem, and 1 *E. coli* isolate (0.9%) had intermediate resistance to imipenem, meropenem and doripenem; It was observed that all strains (100%) were resistant to cefotaxime. *E. coli* isolates are resistant to ampicillin (95.3%), amoxicillin/clavulanic acid (95.3%), cefepime (14.2%), cefixime (19.8%), cephalexin (74.5%), gentamicin (42.5%), kanamycin (37.7%), streptomycin (69.8%), tetracycline (80.2%), ciprofloxacin (60.4%), norfloxacin (13.2%), chloramphenicol (59.4%) and trimethoprim/sulfamethoxazole (68.9%). When we investigated the sequence in the Blast database, the genome of the *E. coli* isolate indicated high similarity with the *mcr*-4 sequences. To our knowledge, this is the first report investigating on the *mcr*-4 gene in *E. coli* identified from cattle in Turkey. Our results highlighted that cattle might be a potential risk in transmitting *mcr* genes.

Keywords: cattle, *Escherichia coli*, *mcr*, multiplex PCR, NGS

© 2024 The Authors. This is an open access article under the CC BY-NC-ND 4.0 license (https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en), which allows re-users to copy and distribute the material in any medium or format in unadapted form and for noncommercial purposes, and only so long as attribution is given to the creator.

Introduction

Antimicrobial resistance which is a global public health problem (WHO 2021), is an inevitable result of the increased and widespread use of antibacterial drugs (Nicolaou and Rigol 2018). The widespread use of antibiotics for human and veterinary therapeutic and prophylactic purposes in animal husbandry has resulted in the global spread of drug-resistant microorganisms (Laxminarayan et al. 2016). The development and worldwide dissemination of antibiotic-resistant organisms have endangered the efficacy of these medications and have reversed advances in antibacterial therapy, and this is limiting treatment decisions (Carvalho and Santos 2016, Kuenzli 2016).

The over-use of colistin represents a serious threat since antibiotic resistance genes can be transmitted from animals to humans (McEachran et al. 2015), and it is anticipated that antibiotic resistance will induce the death of 10 million individuals by 2050 (Luo et al. 2020).

Turkey is reported as being those countries with the highest consumption of antibiotics (TEPAV, 2019). In this context, it is estimated that the Turkish economy may experience a loss of between 220 billion and 1.4 trillion dollars until 2050 due to high antibiotic resistance (TEPAV 2019). Earlier, colistin resistance was thought to be induced only by chromosomal mutations but, in recent years, the emergence and global spread of plasmid-mediated resistance induced by the mobilized colistin resistance (*mcr*) gene have greatly increased (Sun et al. 2017).

The *mcr* gene has been documented in various bacterial species obtained from animal, human, and environmental sources (Wang et al. 2019). The first *mcr*-1 in *E. coli* isolates was detected in animal food and human clinical samples in China in 2015 (Liu et al. 2016). In the following years, different variants related to *mcr* genes appeared in various bacterial species. All *mcr* genes (*mcr*-1−10) (Xavier et al. 2016, AbuOun et al. 2017, Borowiak et al. 2017, Yin et al. 2017, Carretto et al. 2018, Yang et al. 2018, Carroll et al. 2019, Wang et al. 2018, Wang et al. 2020) have been documented in animal samples, while *mcr*-6 and *mcr*-7 have not yet been found in humans (Luo et al. 2020).

Among the beta-lactams, carbapenems are the most effective antibiotics used in Gram-negative bacterial infections (Otlu et al. 2018). Carbapenemases are divided into molecular classes A, B, and D β-lactamases; classes A and D contain β-lactamases with a serine amino acid in their active site, whereas B β-lactamases are metalloenzymes with zinc in the active site (Queenan and Bush 2007). Oxacillin-hydrolyzing (OXA)-48 belonging to class D carbapenemase is encoded by plasmid and is of great interest owing to its difficulty of detection and failure in treatment (Gurung et al. 2020).

NDM-1 enzyme, which is among the class B metallo--beta-lactamases (MBL), was found in bacteria (*K. pneumoniae* and *E. coli*) obtained from a Swedish patient hospitalized in New Delhi in 2009 (Yong et al. 2009). The 2 most common MBL strains found in Enterobacteriales are Verona integron – encoded metallo--beta-lactamase (VIM) and Imipenem (IMP) strains (Cornaglia et al. 2007). The NDM-1-carrying plasmid has generally been associated with *E. coli* but has also been found in *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Salmonella* species (Yong et al. 2009). There is an increase in studies documenting the rapid release of carbapenemase, containing $bla_{\text{VIM-1}}$, $bla_{\text{NDM-1}}$, and $bla_{\text{OXA-48}}$ among CREs in humans (Al Bayssari et al. 2015, Braun et al. 2016, Liu et al. 2016), but not in animals. CRE is still rare (Patel and Bonomo, 2013). The co-occurrence and global spread of plasmid--mediated colistin resistance (*mcr*-1) and carbapenem resistance in Gram-negative bacteria, especially among members of Enterobacterales, can be disastrous (Patel and Bonomo 2013, Skov and Monnet 2016).

The key benefits of the MinION™ are its highly portable and smaller size, easy and fast library preparation, longer sequencing reads, and flexible run time (Kilianski et al. 2015). Owing to the rapid propagation of colistin resistance globally and the detection of isolates containing different resistance genes, it is of great importance to urgently address antimicrobial resistance genes in the national and international areas. However, studies conducted to represent the presence of *mcr* that cause plasmid-mediated colistin resistance, the major carbapenemase ($bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, bla_{IMP}, $_{\text{blaVIM}}$ and bla_{KPC}), β-lactamase ($bla_{\text{TEM-1}}$, $bla_{\text{CTX-M}}$ and $bla_{\text{SHV-1}}$) and OXA-48 like β- lactamase ($bla_{\text{OXA-162}}$, $bla_{\text{OXA-163}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-204}}$ and $bla_{\text{OXA-232}}$) between human and animals in our country are very limited.

In this study, our aims were (1) to search for the existence of bacteria from cattle samples using biochemical methods and Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF), (2) to investigate the occurrence and resistance of phenotypes *E. coli* isolates using the Kirby-Bauer disk diffusion method and to define the minimal inhibitory concentration (MIC) of colistin by broth microdilution, to detect colistin (*mcr*) resistance genes using the multiplex PCR method, (3) to assess the availability of plasmid-mediated colistin (*mcr*-1-9), a carbapenemase ($bla_{\text{OXA-48}}$, bla_{KPC}, bla_{NDM}, bla_{IMP}, and bla_{VIM}), OXA-48-like β-lactamase ($bla_{\text{OXA-162}}$, $bla_{\text{OXA-163}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-204}}$ and $bla_{\text{OXA-232}}$) and β-lactamase (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$) resistance genes in *E. coli* isolates using multiplex PCR, (4) to detect the genetic characteristics

of isolates harboring *mcr* genes using Next-generation sequencing (NGS) – PCR Amplicons with Nanopore Technology.

Materials and Methods

Ethical approval

This study was performed based on ethical standards and validated by the Bingol University Ethics Committee under protocol number E.34805, dated 22.10.2021.

Sampling and identification of *E. coli*

In this study, a total of 240 fresh fecal samples taken directly from each cow's rectum were collected from 12 private farms in the Bingol province of East Turkey from November 2021 to January 2022 at different intervals. Fecal samples were taken in a sterile stool container to a cool box containing ice and immediately transferred to the microbiological laboratory for analysis. The swabs were aseptically inoculated into 10 ml Tryptic Soy broth (enrichment broth) (Oxoid, Basingstoke, UK) and incubated at 37°C for 18-24 hours under aerobic conditions. One loopful culture of the pre-enriched liquid was plated on MacConkey agar (Merck, Germany) and Eosin Methylene Blue (EMB) agar (Merck, Germany), and incubated at 37°C for 24 hours. Following this, lactose-positive (pink color) on MacConkey agar and colonies that gave a metallic green reflective color on EMB agar were considered suspicious for *E. coli* (Bhoomika et al. 2016). The suspected *E. coli* colonies were identified by biochemical methods and the MALDI-TOF system (bioMerieux, France) (Dubois et al. 2012, Westblade et al. 2013). All *E. coli* positive samples were searched for *mcr-1* to *mcr-9* using multiplex PCR and the *mcr*-positive isolates were further confirmed by NGS – PCR Amplicons with Nanopore Technology.

Multiplex PCR

Antibiotic resistance genes were detected using multiplex PCR (Table 1) (Borowiak et al. 2020, Ellington et al. 2007, Hasman et al. 2005, Poirel et al. 2011, Rebelo et al. 2018). Genomic DNA was detected on a QIAsymphony instrument using the QIAsymphony DSP Virus / Pathogen midi kit (Qiagen, Hilden, Germany) following by the manufacturer's instruction. The genomic DNA was enhanced by multiplex PCR utilizing primers specific to *mcr*-1-9 resistance genes (Table 1) (Rebelo et al. 2018, Borowiak et al. 2020).

Each PCR reaction included 12.5 µL *TopTaq* Master Mix (Qiagen), 5.5 µL of nuclease-free water, 0.5 µL of each of the primer solutions (10 µM), and 2 µL template DNA. PCR conditions were: pre-denaturation at 94°C for 15 min, induced by 25 cycles of denaturation at 94°C for 30 s, 90 s of annealing step a 58°C, 60 s of elongation step at 72°C, and a final extension at 72°C for 10 min (Hasman et al. 2005, Ellington et al. 2007, Poirel et al. 2011, Rebelo et al. 2018, Borowiak et al. 2020). The amplification was visualised using agarose gel electrophoresis followed by ethidium-bromide staining. PCR products were monitored using a gel imaging system (Fig. 1). *E. coli* 2012–60–1176–27 for *mcr*-1, IncX4 plasmid for *mcr*-2, 2013-SQ352 for *mcr*-3, DH5α pCR2 for *mcr*-4, *Salmonella Paratyphi* B (13-SA01718 for *mcr*-5) as positive control (provided by Associate Professor Mehmet Cemal Adiguzel, Department of Microbiology, Faculty of Veterinary Medicine, Ataturk University, Turkey), the major carbapenemase and β-lactamase genes harboring *E. coli* strains as positive control (provided by Professor Baris Otlu, Department of Medical Microbiology, Faculty of Medicine, Inonu University, Malatya, Turkey) were used in all assays.

Determination of colistin MIC by broth microdilution method

Escherichia coli isolates were detected using the broth microdilution method as suggested by the European Committee for Antimicrobial Susceptibility Tests (EUCAST, 2021). The MIC value, which is defined as the lowest colistin concentration without growth in the medium, was calculated. According to EUCAST criteria, isolates with a MIC of > 2 mg/L for Enterobacterales were accepted as resistant (EUCAST 2021). *E. coli* ATCC 25922 was used as a positive control strain.

Antimicrobial susceptibility assays

Antimicrobial susceptibilities of *E. coli* were detected using the Kirby-Bauer disk diffusion method as described by EUCAST (2021). The suspension adjusted to the 0.5 McFarland standard was streaked evenly on a Mueller-Hilton agar plate (Oxoid, England). Commercial antibiotic discs with amikacin (AMK, 30 µg), ampicillin (AMP, 10 µg), tetracycline (TET, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), streptomycin (STR, 10 µg), cefoxitin (FOX, 30 µg), cefixime (CFM, 5 µg), cephalexin (LEX, 30 µg), gentamicin (GEN, 30 µg), kanamycin (KAN, 30 µg), ertapenem (ETP, 30 µg), imipenem (IMP, 30 µg), meropenem (MEM, 30 µg), chloramphenicol (CHL, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), streptomycin (STR, 30 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), cefepime (FEP, 30 µg), norfloxacin (NOR, 10 µg), doripenem

Table 1. Primers used for determining the plasmid-mediated antimicrobial resistance genes in *Escherichia coli* isolates.

Primers	Primer sequences (5′-3′)	Genes	The size of PCR products (bp)	References
mcr-1_320bp_fw	AGTCCGTTTGTTCTTGTGGC	*mcr*-1	320	Rebelo et al. 2018
mcr-1_320bp_rev	AGATCCTTGGTCTCGGCTTG			
mcr-2_700bp_fw	CAAGTGTGTTGGTCGCAGTT	*mcr*-2	715	Rebelo et al. 2018
mcr-2_700bp_rev	TCTAGCCCGACAAGCATACC			
mcr-3_900bp_fw	AAATAAAAATTGTTCCGCTTATG	*mcr*-3	929	Rebelo et al. 2018
mcr-3_900bp_rev	AATGGAGATCCCCGTTTTT			
mcr-4_1100bp_fw	TCACTTTCATCACTGCGTTG	*mcr*-4	1116	Rebelo et al. 2018
mcr-4_1100bp_rev	TTGGTCCATGACTACCAATG			
*MCR*5_fw	ATGCGGTTGTCTGCATTTATC	*mcr*-5	1644	Borowiak et al. 2017
*MCR*5_rev	TCATTGTGGTTGTCCTTTTCTG	bla_{TEM-1}	931	Ellington et al. 2007
mcr-6_mp_fw	AGCTATGTCAATCCCGTGAT	*mcr*-6	252	Borowiak et al. 2020
mcr-6_mp_rev	ATTGGCTAGGTTGTCAATC			
mcr-7_mp_fw	GCCCTTCTTTTCGTTGTT	*mcr*-7	551	Borowiak et al. 2020
mcr-7_mp_rev	GGTTGGTCTCTTTCTCGT			
mcr-8_mp_fw	TCAACAATTCTACAAAGCGTG	*mcr*-8	856	Borowiak et al. 2020
mcr-8_mp_rev	AATGCTGCGCGAATGAAG			
mcr-9_mp_fw	TTCCCTTTGTTCTGGTTG	*mcr*-9	1011	Borowiak et al. 2020
mcr-9_mp_rev	GCAGGTAATAAGTCGGTC			
NDM-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM}	621	Poirel et al. 2011
NDM-R	CGGAATGGCTCATCACGATC			
KPC-F	CGTCTAGTTCTGCTGTCTTG	bla_{KPC}	798	Poirel et al. 2011
KPC-R	CTTGTCATCCTTGTTAGGCG			
OXA-48-F	GCGTGGTTAAGGATGAACAC	bla_{OXA-48}	438	Poirel et al. 2011
OXA-48-R	CATCAAGTTCAACCCAACCG			
OXA-48A-F	TTGGTGGCATCGATTATCGG	bla_{OXA-48}-like	743	Poirel et al. 2010
OXA-48B-R	GAGCACTTCTTTTGTGATGGC			
IMP-F	GGAATAGAGTGGCTTAAYTCTC	bla_{IMP}	232	Poirel et al. 2011
IMP-R	GGTTTAAYAAAACAACCACC			
VIM-F	GATGGTGTTTGGTCGCATA	bla_{VIM}	390	Poirel et al. 2011
VIM-R	CGAATGCGCAGCACCAG			
TEM-F	TCCGCTCATGAGACAATAACC	bla_{TEM}	931	Sturenburg et al. 2004
TEM-R	TTGGTCTGACAGTTACCAATGC			
S1-F	TGGTTATGCGTTATATTCGCC	bla_{SHV}	868	Pai et al. 1999
S2-R	GGTTAGCGTTGCCAGTGCT			
CTX-M-F	ATGTGCAGYACCAGTAARGTKATGGC	bla_{CTX-M}	593	Hasman et al. 2005
CTX-M-R	TGGGTRAARTARGTSACCAGAAYCAGCGG			

(DOR, 10 µg) and amoxicillin/clavulanic acid (AMC, 20/10 µg) were placed over the Mueller-Hinton agar plate and incubated at 37°C for 18-24 hours (Hassan et al. 2020). Results were recorded by measuring the diameters of the zone of inhibition and the results were interpreted according to the EUCAST standards (EUCAST 2021).

Statistical analysis

The SPSS 26.0 package program was used in the statistical analysis of the data. The chi-square test or Fisher's exact test, when appropriate, was used to compare antibiotic sensitivity rates and the value of $p<0.05$ was considered statistically significant.

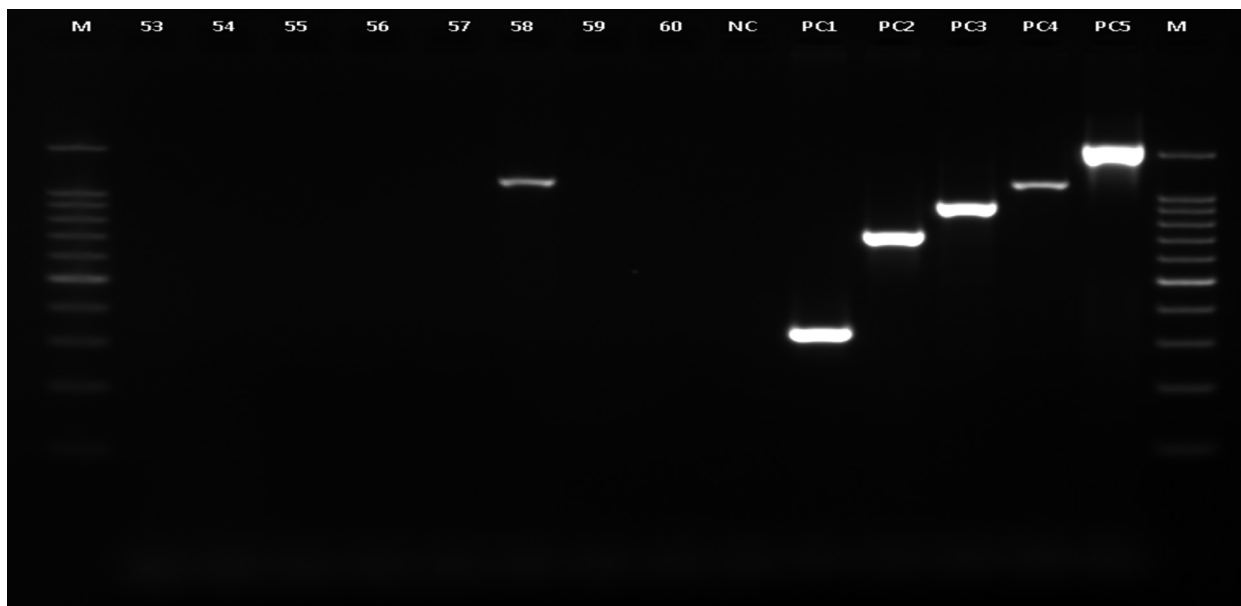

Fig. 1. Agarose gel (1.5%) electrophoresis of multiplex PCR amplification products. Lanes labeled NC: negative control, PC-1: *mcr*-1 positive control (320 bp), PC-2: *mcr*-2 (715 bp) positive control, PC-3: *mcr*-3 (929 bp) positive control, PC-4: *mcr*-4 (1116 bp) positive control, PC-5: *mcr*-5 (1644 bp) positive control, 58: *mcr*-4 (1116 bp) isolates from cattle, M: DNA Ladder (100 bp) (Transgen Biotech, Beijing, China).

NGS – PCR amplicons with Nanopore Technology

Next-generation sequencing of PCR amplicon samples was performed with Oxford Nanopore Technologies (ONT, Oxford, UK) using a MinION™ instrument. The key benefits of the MinION™ are its highly portable and smaller size, easy and fast library preparation, longer sequencing reads, and flexible run time (Kilianski et al. 2015). In sequencing, the obtained PCR products were purified using the AMPureXP, magnetic particle method (Thermo Fisher, MA, USA) to prepare DNA libraries for sequencing. The purity and actual concentration of the amplicons were measured using NanodropOne (Thermo Fisher) and Qubit2.0 (Thermo Fisher), respectively. These samples between DNA 260/280 ~1.80 and 260/230 ~2.0-2.2 purities and concentrations accepted by ONT, were barcoded using the transposons mechanism using a ONT SQK-RBK004 kit and put into the DNA pool for multiplex sequencing. While preparing the loading library, ONT sequencing buffer and loading particles were added outside the DNA pool, and was made ready for sequencing in the MinION Mk1B platform using R9.4.1 flow cells.

Data were then obtained as fast5 files using the MinKNOW (v. 21.10.4) interface, electrical signals were converted to organic base sequences with ONT Guppy (v.5.0.16), and the strings and adapter sequences of the SQK-RBK004 kit used were removed. Using the FastQC (v. 0.9.6) CLI program, the read lengths of the sequences and the quality scores per base (phred scores) were calculated. The average observed accuracy for R9.4.1 reads was determined as corresponding to a Phred score of Q17. The barcodes of multiplex sequencing were then removed using ONT-Guppy.

Results

106 *E. coli* was identified by culture and MALDI--TOF. The 106 *E. coli* isolates were subjected to multiplex PCR to determine the existence of *mcr* genes with the primers specific for *mcr*. The *mcr*-4 gene was found in only one *E. coli* isolate using multiplex PCR (Fig. 1). *mcr*-4 harboring *E. coli* isolate was detected to be resistant to colistin at the same time. In addition, *E. coli* isolate was negative for carbapenemase and OXA-48-like β-lactamase and positive for β-lactamase resistance genes.

E. coli isolates carrying *mcr*-4 were more resistant to colistin. Antimicrobial susceptibility testing using the disk diffusion assay indicated that all 106 *E. coli* isolates (100%) were sensitive to AMK and 105 *E. coli* isolates (99.1%) exhibited sensitivity (S) to IPM, MEM, and DOR and 1 *E. coli* isolate (0.9%) exhibited intermediate (I) resistance (R) to IPM, MEM and DOR; it was observed that all strains (100%) were resistant to CTX. They were also resistant to AMP (95.3%), AMC (95.3%), FEP (14.2%), CFM (19.8%), LEX (74.5%), GEN (42.5%), STR(69.8%), KAN (37.7%), TET (80.2%), CIP (60.4%), NOR (13.2%), CHL (59.4) and trimethoprim/ sulfamethoxazole (68.9%) (Table 2).

Chi-square analysis shows that the level of susceptibility of *E. coli* isolates, obtained from the bovine fresh fecal samples, to antibiotics is strongly related

Table 2. Antibiotic resistance of *Escherichia coli* isolates obtained from bovine fresh fecal samples.

Antibiotics	S	I	R	P value
Penicilins				
AMC	5 (4.7)		101 (95.3)	< 0.001
AMP	5 (4.7)		101 (95.3)	
Cefalosporines				
CTX	-		106 (100)	0.38
FEP	90 (84.9)	1 (0.9)	15 (14.2)	
CFM	85 (80.2)		21 (19.8)	
LEX	26 (24.5)	1 (0.9)	79 (74.5)	
FOX	101 (95.3)		5 (4.7)	
CAZ	22 (20.8)		84 (79.2)	
Carbapenems				
ETP	91 (85.8)		15 (14.2)	< 0.001
IMP	105 (99.1)	1 (0.9)	-	
DOR	105 (99.1)	1 (0.9)	-	
MEM	105 (99.1)	1 (0.9)	-	
Aminoglycosides				
AMK	106 (100%)		-	0.31
GEN	60 (56.6)	1 (0.9)	45 (42.5)	
KAN	65 (61.3)	1 (0.9)	40 (37.7)	
STR	31 (29.2)	1 (0.9)	74 (69.8)	
Fluoroquinolones				
CIP	40 (37.7)	2 (1.9)	64 (60.4)	0.24
NOR	91 (85.8)	1 (0.9)	14 (13.2)	
Tetracyclines				
TET	21 (19.8)		85 (80.2)	< 0.01
Sulfonamides				
SXT	33 (31.1)		73 (68.9)	< 0.01
Other				
CHL	42 (39.6)	1 (0.9)	63 (59.4)	< 0.05

Note: S – sensitive, R – resistant, ampicillin (AMP), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX), cefepime (FEP), cefixime (CFM), cephalexin (LEX), cefoxitin (FOX), ceftazidime (CAZ), ertapenem (ETP), imipenem (IMP), doripenem (DOR), meropenem (MEM), amikacin (AMK), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), tetracycline (TET), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (CHL)

to the type of antibiotic. The results represented that *E. coli* isolates were resistant to penicilins ($p<0.001$) with ampicillin and amoxicillin/clavulanic acid having the highest resistance value. Likewise, significant resistance was confirmed to tetracycline (tetracyclines), trimethoprim-sulfamethoxazole (sulfonamides) and chloramphenicol ($p<0.01$). In contrast, the high sensitivity of *E. coli* isolates to carbapenems including ertapenem, imipenem, doripenem and meropenem was confirmed ($p<0.001$). Statistical significance was not confirmed ($p>0.05$) for antibiotics from the groups cephalosporins, aminoglycosides and fluoroquinolones (Table 2).

Screening of the *mcr*-4 (Phosphoethanolamine-lipid A transferase) gene amplicon, which has a length of 1116 bp generated from the culture of *E. coli* was performed in the sequencing study. The NCBI BLAST database (http://blast.ncbi.nlm.nih.gov) of the reads were taken and the readings matched the *E. coli* bacterial strain with high similarity.

Discussion

According to a Chinese study, the prevalence of colistin-resistant *E. coli* in cattle was low (0.9%), whereas the rates of resistance in *E. coli* from poultry and pigs were higher (14% and 24%, respectively) (Huang et al. 2017). Several sources have led Denmark and other nations to report the global dissemination

of this gene (Hasman et al. 2015, Skov and Monnet 2016).

In China during 2015–2016, the proportion of *mcr*-1-carrying *E. coli* has been documented to be 71.43, 68.86, and 87.58% from cattle, swine, and chickens, respectively (Zhang et al. 2019) and 20.43%, 8.47% and 2.97% in Japan from swine and cattle, respectively (Nakano et al. 2021). A study has indicated the availability of *mcr*-1 in an *E. coli* isolate from a cow with subclinical mastitis, despite a very low prevalence in Egypt (Khalifa et al. 2016). *mcr*-1 so far been the most dominant gene in animals (Hasman et al. 2015, Liu et al. 2016, Hernandez et al. 2017, Haenni et al. 2018). The *mcr*-2 gene was demonstrated in *E. coli* from cattle and pigs in Belgium (Xavier et al. 2016). There are only a few reports indicating the presence of *mcr*-2: 21% of the isolates of *E. coli* that generate extended-spectrum beta-lactamase (ESBL) were taken from calves (Haenni et al. 2018), 46.82%, 14.90%, and 19.05% of the colistin-resistant *E. coli* that were taken from fecal swabs came from pigs, chickens, and cattle, respectively (Zhang et al. 2019). Other studies showed no *mcr*-2 in *E. coli* isolates from broilers, cattle, and swine, whereas the prevalence of *mcr*-1 was slightly raised (Kawanishi et al. 2017, Zając et al. 2019). *Mcr*-3 in *E. coli* isolates has been indicated by Hernandez et al. (2017) in cattle. We reported the *mcr*-4 gene in *E. coli* as well as the phenotypic resistance. *mcr*-4 was first found in *Salmonella enterica* serovar Typhimurium in a pig in Italy (Carattoli et al. 2017), subsequently, *mcr*-4 was demonstrated in *Salmonella enterica* serovar Typhimurium from humans with gastroenteritis (Carretto et al. 2018). In 2014 and 2015, an Italian study on the epidemiology of colistin-resistant *Salmonella* isolates and *E. coli* isolates in bovines reported that the majority of colistin-resistant isolates were shown to harbor *mcr*-1 genes. On the other hand, *mcr*-3 genes were described in *E. coli* isolates from bovines that produced ESBL, and two *mcr*-4 genes were found in *E. coli* isolate from pigs and bovines and an ESBL. Furthermore, the *mcr*-3.2 and *mcr*-4.3 genes were found in *E. coli* from bovines, while *mcr*-4.2 was found in *E. coli* from pigs (Alba et al. 2018).

In Turkey, antibiotics are used intensively and without restraint for the treatment of infectious diseases in farm animals, and sometimes they are added to water or feed to protect the animals and increase growth. Therefore, food animals are an important reservoir for the transfer of antibiotic-resistant bacteria. This situation adversely affects human and animal health and harms the country's economy. While there are very few clinical studies on antimicrobial resistance in our country, community, agricultural and environmental studies are insufficient, and it is estimated that a very important part of antimicrobial resistance is due to antimicrobials used in livestock (TEPAV, 2019). There are few studies in Turkey to reveal the existence of plasmid-mediated colistin resistance genes between humans and animals. In two studies conducted in Turkey, plasmid-mediated *mcr* gene positivity was not reported in human and animal isolates (Sari et al. 2017, Duman and Tekerekoğlu 2020). In addition, in our country, the existence of *mcr* genes has also been reported in *E. coli* strains from humans (Özkaya et al. 2020), chicken meat (Kurekci et al. 2018, Adigüzel et al. 2021), cattle and sheep (Ayaz et al. 2019) and mastitis milk (Babacan 2023). Four *mcr*-positive *E. coli* strains (A1, A5, A7, and A9) were identified from 80 chicken meats from local butchers/markets in Adana and Hatay provinces, and this study is the first *mcr* study of animal origin in Turkey (Kurekci et al. 2018). Moreover, Ayaz et al. (2019) notified the first occurrence of plasmid-mediated *mcr*-2 and *mcr*-3 genes in *E. coli* O157:H7 isolated from cattle and sheep in another study conducted in our nation. Only one of the 11 isolates from retail raw chicken meat-carried the *mcr*-1 gene, according to a study conducted in eastern Turkey between 2016 and 2019 that examined colistin resistance in 500 *E. coli* isolates isolated from various food and animal sources (Adiguzel et al. 2021). In addition, as a result of whole genome sequence analysis, it was determined that the isolate named ATAVET *mcr*-1 Turkey isolate (Adiguzel et al. 2022). In a study using the PCR method, of the colistin resistance of 48 *E. coli* isolates isolated from food samples in Turkey between 2011 and 2015, the *mcr* gene was not found in any of the multidrug-resistant isolates (Guzel et al. 2020). In the bacteriological examination of 212 milk samples obtained from Balikesir province, 14 (6.60%) *E. coli*, 3 (1.41%) *K. oxytoca*, and 2 (0.94%) *K. pneumonia* were isolated, and of the 2 *E. coli* isolates were resistant to antibiotics. The study, which reported that *K. oxytoca* isolates were resistant to all of them and that *K. oxytoca* isolates had the highest resistance to cefotaxime and amoxicillin, is the first to report *mcr* and carbapenem resistance genes obtained from dairy cows with mastitis in Turkey (Babacan 2023). In the current study, *mcr*-4 harboring *E. coli* was determined in only one isolate by multiplex PCR. These data match the low proportion of *mcr* genes in animal and human clinical isolates in earlier studies that were reported in Turkey.

Conclusion

This is the first report that we are aware of regarding *E. coli* that harbors the *mcr*-4 gene, which was identified from cattle fecal samples in Turkey. The results

of this investigation demonstrate that cows are an important source of colistin-resistant *E. coli*. It is known that the dissemination of these antibiotic-resistance genes may be faster than expected, and their investigation in animals is of urgent importance. The identification of plasmid-mediated *mcr*-4 genes in this study will contribute to future risk assessment policies on colistin--resistant *E. coli*.

Since the spread of antibiotic resistance genes is faster than expected, resistance genes can be transmitted from animals to humans, and there are risks that it may cause a global epidemic in the near future, it is of urgent importance to investigate this critical issue in animal, human and environmental sources within the framework of the One Health approach.

Therefore, to curb the dissemination of antibiotic resistance in animals, human and the environment in Turkey, it is imperative to establish reliable monitoring systems and take appropriate action.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0457 and project VEGA no. 1-0162-23: *Reduction in antibiotic use in dairy mastitis control programs*.

References

AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, NunezGarcia J, Randall LP, Lemma F, Crook DW, Teale C, Smith RP, Anjum MF **(2017)** mcr-1 and mcr-2 variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother 72: 2745-2749.

Adiguzel MC, Baran A, Wu Z, Cengiz S, Dai L, Oz C, Ozmenli E, Goulart DB, Sahin O **(2021)** Prevalence of colistin resistance in *Escherichia coli* in eastern Turkey and genomic characterization of an *mcr-1* positive strain from retail chicken meat. Microb Drug Resist 27: 424-432.

Alba P, Leekitcharoenphon P, Franco A, Feltrin F, Ianzano A, Caprioli A, Stravino F, Hendriksen RS, Bortolaia V, Battisti A **(2018)** Molecular epidemiology of mcr-encoded colistin resistance in Enterobacteriaceae from food-producing animals in Italy revealed through the EU harmonized antimicrobial resistance monitoring. Front Microbiol 9: 1217.

Al-Bayssari C, Dabboussi F, Hamze M, Rolain JM **(2015)** Detection of expanded-spectrum β-lactamases in Gram--negative bacteria in the 21st century. Expert Rev Anti Infect Ther 13: 1139-1158.

Ayaz ND, Cufaoglu G, Yonsul Y, Goncuoglu M, Erol I **(2019)** Plasmid-mediated colistin resistance in *Escherichia coli* O157:H7 cattle and sheep isolates and whole-genome sequence of a colistin-resistant sorbitol fermentative *Escherichia coli* O157:H7. Microb Drug Resist 25: 1497-1506.

Babacan O **(2023)** First detection of carbapenem resistance in Enterobacteriaceae isolates isolated from dairy cows' mastitis infection in Türkiye. Ankara Univ Vet Fak Derg 70: 65-74.

Bhoomika SS, Patyal A, Gade NE **(2016)** Occurrence and characteristics of extended-spectrum β-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. Vet World 9: 996-1000.

Borowiak M, Baumann B, Fischer J, Thomas K, Deneke C, Hammerl JA, Szabo I, Malorny B **(2020)** Development of a novel *mcr-6* to *mcr-9* multiplex PCR and Assessment of *mcr-1* to *mcr-9* occurrence in colistin-resistant *Salmonella enterica* isolates. From environment, feed, animals and food (2011-2018) in Germany. Front Microbiol 11: 80.

Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B **(2017)** Identification of a novel transposon--associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. enterica serovar Paratyphi B. J Antimicrob Chemother 72: 3317-3324.

Braun SD, Ahmed MFE, El-Adawy H, Hotzel H, Engelmann I, Weiß D, Monecke S, Ehricht R **(2016)** Surveillance of extended-spectrum beta-lactamase-producing *Escherichia coli* in dairy cattle farms in the Nile Delta, Egypt. Front Microbiol 7: 1020.

Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF **(2017)** Novel plasmid-mediated colistin resistance mcr-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 22: 30589.

Carretto E, Brovarone F, Nardini P, Russello G, Barbarini D, Pongolini S, Gagliotti C, Carattoli A, Sarti M **(2018)** Detection of mcr-4 positive Salmonella enterica serovar Typhimurium in clinical isolates of human origin, Italy, October to November 2016. Eur Surveill 23: 17-00821.

Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M **(2019)** Identification of novel mobilized colistin resistance gene mcr-9 in a multidrugresistant, colistin-susceptible Salmonella enterica serotype Typhimurium isolate. mBio 10: e00853-19.

Carvalho IT, Santos L **(2016)** Antibiotics in the aquatic environments: a review of the European scenario. Environ Int 94: 736-757.

Cornaglia G, Akova M, Amicosante G, Cantón R, Cauda R, Docquier JD, Edelstein M, Frère JM, Fuzi M, Galleni M, Giamarellou H, Gniadkowski M, Koncan R, Libisch B, Luzzaro F, Miriagou V, Navarro F, Nordmann P, Pagani L, Peixe L, Poirel L, Souli M, Tacconelli E, Vatopoulos A, Rossolini GM; ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS) **(2007)** Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents 29: 380-388.

Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E **(2012)** Performances of the Vitek MS matrix-assisted laser desorption ionization – time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J Clin Microbiol 50: 2568-2576.

Duman ve Tekerekoğlu A **(2020)** Colistin MICs and resistance genes of *Acinetobacter Baumannii* ısolated in ıntensive care units. Turk J Intensive Care DOI: 10.4274/tybd.galenos.2020.47965.

Ellington MJ, Kistler J, Livermore DM, Woodford N **(2007)** Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 59: 321-322.

EUCAST, European Committee on Antimicrobial Susceptibility Testing **(2021)** Breakpoint tables for ınterpretation of MICs and zone diameters, EUCAST, Version 11.1. https://www.eucast.org/mic_and_zone_distributions_and_ecoffs

Gurung S, Kafle S, Dhungel B, Adhikari N, Thapa Shrestha U, Adhikari B, Banjara MR, Rijal KR, Ghimire P **(2020)** Detection of OXA-48 gene in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from urine smples. Infect Drug Resist 13: 2311-2321.

Güzel M, Avşaroğlu MD, Soyer Y **(2020)** Determination of colistin resistance in *Escherichia coli* isolates from foods in Turkey, 2011-2015. Food Health 6: 160-169.

Haenni M, Beyrouthy R, Lupo A, Chatre P, Madec JY, Bonnet R **(2018)** Epidemic spread of *Escherichia coli* ST744 isolates carrying mcr-3 and blaCTX- M-55 in cattle in France. J Antimicrob Chemother 73: 533-536.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM **(2005)** beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother 56: 115-121.

Hassan J, Eddine RZ, Mann D, Li S, Deng X, Saoud IP, Kassem II **(2020)** The mobile colistin resistance gene, mcr-1.1, ıs carried on IncX4 plasmids in multi-drug resistant *E. coli* ısolated from rainbow trout aquaculture. Microorganisms 23: 1636.

Hernandez M, Iglesias MR, Rodriguez-Lázaro D, Gallardo A, Quijada N, Miguela-Villoldo P, Campos MJ, Piriz S, Lopez-Orozco G, de Frutos C, Saez JL, Ugarte-Ruiz M, Dominguez L, Queseda A **(2017)** Co-occurrence of colistin--resistance genes mcr-1 and mcr-3 among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. Euro Surveill 22: 30586.

Huang X, Yu L, Chen X, Zhi C, Yao X, Liu Y, Wu S, Guo Z, Yi L, Zeng Z, Liu JH **(2017)** High prevalence of colistin resistance and *mcr-1* gene in *Escherichia coli* isolated from food animals in China. Front Microbiol 8: 562.

Kawanishi M, Abo H, Ozawa M, Uchiyama M, Shirakawa T, Suzuki S, Shima A, Yamashita A, Sekizuka T, Kato K, Kuroda M, Koike R, Kijimaet M **(2017)** Prevalence of colistin resistance gene *mcr-1* and absence of *mcr-2* in *Escherichia coli* isolated from healthy food-producing animals in Japan. Antimicrob Agents Chemothe 61: e02057-16.

Khalifa HO, Ahmed AM, Oreiby AF, Eid AM, Shimamoto T, Shimamoto T **(2016)** Characterisation of the plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* isolated from animals in Egypt. Int J Antimicrob Agents 47: 413-414.

Kilianski, A, Haas JL, Corriveau EJ, Liem AT, Willis KL, Kadavy DR, Rosenzweig CN, Minot SS **(2015)** Bacterial and viral identification and differentiation by amplicon sequencing on the MinION nanopore sequencer. Gigascience 4: 12.

Kuenzli E **(2016)** Antibiotic resistance and international travel: causes and consequences. Travel Med Infect Dis 14: 595-598.

Kurekci C, Aydin M, Nalbantoglu OU, Gundogdu A **(2018)** First report of *Escherichia coli* carrying the mobile colistin resistance gene mcr-1 in Turkey. J Glob Antimicrob Resist. 15: 169-170.

Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, Davies S **(2016)** Access to effective antimicrobials: a worldwide challenge. Lancet 387: 168-175.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J **(2016)** Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16: 161-168.

Luo Q, Wang Y, Xiao Y **(2020)** Prevalence and transmission of mobilized colistin resistance (mcr) gene in bacteria common to animals and humans. Biosaf Health 2: 71-78.

McEachran AD, Blackwell BR, Hanson JD, Wooten KJ, Mayer GD, Cox SB, Smith PN **(2015)** Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environ Health Perspect 123: 337-343.

Nakano A, Nakano R, Nishisouzu R, Suzuki Y, Horiuchi S, Kikuchi-Ueda T, Ubagai T, Ono Y, Yano H **(2021)** Prevalence and Relatedness of *mcr-1*-Mediated colistin-resistant *Escherichia coli* ısolated from livestock and farmers in Japan. Front. Microbiol 12: 664931.

Nicolaou KC, Rigol S **(2018)** A brief history of antibiotics and select advances in their synthesis. J Antibiot (Tokyo) 71: 153-184.

Otlu B, Yakupoğulları Y, Gürsoy NC, Duman Y, Bayındır Y, Tekerekoğlu MS, Ersoy Y (2018) Co-production of OXA-48 and NDM-1 Carbapenemases in Providencia rettgeri: the first report. Mikrobiyol Bul 52: 300-307.

Özkaya E, Buruk CK, Tosun İ, Toraman B, Kaklıkkaya N, Aydın F **(2020)** Investigation of plasmid mediated *mcr* colistin resistance gene in clinical Enterobacterales isolates. Mikrobiyol Bul 54:191-202.

Pai H, Lyu S, Lee JH, Kim J, Kwon Y, Kim JW, Choe KW **(1999)** Survey of extended-spectrum β-lactamases in clinical isolates of Escherichia coli and Klebsiella pneumoniae: prevalence of TEM-52 in Korea. J Clin Microbiol 37: 1758-1763.

Patel G, Bonomo RA **(2013)** Stormy waters ahead: global emergence of carbapenemases. Front Microbiol 4: 48.

Poirel L, Naas T, Nordmann P **(2010)** Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54: 24-38.

Poirel L, Walsh TR, Cuvillier V, Nordmann P **(2011)** Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119-123.

Queenan AM, Bush K **(2007)** Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 20: 440-458.

Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS **(2018)** Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* for surveillance purposes. Euro Surveill 23: 17-00672.

Sarı AN, Süzük S, Karatuna O, Öğünç D, Karakoç AE, Çizmeci Z, Alışkan HE, Cömert F, Bakıcı MZ, Akpolat N, Çilli FF, Zer Y, Karataş A, Akgün Karapınar B, Bayramoğlu G, Özdamar M, Kalem F, Delialioğlu N, Aktaş E, Yılmaz N, Gürcan S, Gülay Z **(2017)** Results

of a multicenter study ınvestigating plasmid mediated colistin resistance genes (mcr-1 and mcr-2) in clinical Enterobacteriaceae ısolates from Turkey. Mikrobiyol Bul 51: 299-303.

Skov RL, Monnet DL **(2016)** Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Eurosurveill 21: 30155

Sturenburg E, Kühn A, Mack D, Laufs R **(2004)** A novel extended-spectrum β-lactamase CTX-M-23 with a P167T substitution in the active-site omega loop associated with ceftazidime resistance. J Antimicrobial Chemother 54: 406-409.

Sun J, Zeng X, Li XP, Liao XP, Liu YH, Lin J **(2017)** Plasmid-mediated colistin resistance in animals: current status and future directions. Anim Health Res Rev 18: 136-152.

TEPAV **(2019)** Antimicrobial resistance in Turkey: Economic evaluation and recommendations. TEPAV: 1-30. https://www.tepav.org.tr/upload/files/1504774735-1.Turkiye'de Antimikrobiyal Direnç Ekonomik Degerlendirme ve Oneriler.pdf.

Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z **(2020)** Identification of novel mobile colistin resistance gene mcr-10. Emerg Microb Infect 9: 508-516.

Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A **(2018)** The global distribution and spread of the mobilized colistin resistance gene mcr-1, Nat Communi 9: 1179.

Wang X, Wang Y, Zhou Y, Wang Z, Wang Y, Zhang S, Shen Z **(2019)** Emergence of colistin resistance gene mcr-8 and its variant in *Raoultella ornithinolytica*. Fron Microbiol 10: 228.

Westblade LF, Jennemann R, Branda JA, Bythrow M, Ferraro MJ, Garner OB, Ginocchio CC, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Rychert JA, Sercia L, Burnham CA **(2013)** Multicenter study evaluating the Vitek MS system for identification of medically important yeasts. J Clin Microbiol 51: 2267-2272.

WHO. World Health Organization **(2021)** Antibiotic resistance, https://www.who.int/news-room/fact-sheets/detail/antibioticresistance.

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S **(2016)** Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Eurossurveill 21: 30280.

Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN **(2018)** Novel plasmid-mediated colistin resistance gene mcr-7.1 in *Klebsiella pneumonia*. J Antimicrob Chemother 73: 1791-1795.

Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y **(2017)** Novel plasmid-mediated colistin resistance gene mcr-3 in *Escherichia coli*. mBio 8: e00543-17.

Yong JW, Ge L, Ng YF, Tan SN **(2009)** The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14: 5144-5164.

Zając M, Sztromwasser P, Bortolaia V, Leekitcharoenphon P, Cavaco LM, Ziętek-Barszcz A, Hendriksen RS, Wasyl D **(2019)** Occurrence and characterization of *mcr-1*-positive *Escherichia coli* isolated from food-producing animals in Poland, 2011-2016. Front Microbiol 10: 1753.

Zhang X, Zhang B, Guo Y, Wang J, Zhao P, Liu J, He K **(2019)** Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu Province. Int J Food Microbiol 291: 87-90.