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Original article

Antimicrobial resistance patterns and high prevalence of *the mcr-1* gene in pathogenic *Escherichia coli* from diseased poultry

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Abstract

This study assessed the prevalence of the *mcr-1* gene, which provides resistance to colistin (polymyxin E), in *Escherichia coli* strains isolated from sick poultry in the Peshawar, Mardan, and Charsadda regions of Pakistan. Samples were collected from 246 poultry birds showing symptoms such as diarrhoea, respiratory distress, and other systemic infections. After enrichment in Brain Heart Infusion broth, the samples were cultured on selective media containing colistin. The isolated colonies were further cultured on Eosin Methylene Blue agar and analyzed with PCR to detect the *mcr-1* gene. The study found varying levels of *mcr-1* gene prevalence across the three regions, with Charsadda exhibiting the highest frequency. Antibiogram analysis showed high resistance to ampicillin, colistin, and sulfamethoxazole, and low resistance to tetracycline, norfloxacin and cefixime. In conclusion, the findings suggest that colistin resistance exists in *E. coli* from poultry in these regions, with notable resistance trends to commonly used antibiotics such as ampicillin and sulfamethoxazole. Consequently, these antibiotics should be avoided for treating *E. coli* infections, and alternatives like ceftazidime and cefepime should be considered. This research emphasizes the importance of ongoing monitoring of antibiotic resistance in poultry pathogens and the need for more tar-geted treatment approaches.

Keywords: colistin resistance, *mcr-1* gene, *E. coli*, antibiogram, antibiotics, poultry

Introduction

Antimicrobial resistance is an increasing global health threat, especially in the poultry industry, leading to significant economic losses (Sarmah et al. 2006). The widespread use of antibiotics as growth promoters has contributed to the rise of drug-resistant bacteria (Munday et al. 2014). Colistin, a last-resort antibiotic from the polymyxin E group, disrupts the lipopolysaccharide layer in bacterial cell membranes, resulting in bacterial death (Moffatt et al. 2010). Polymyxins are positively charged peptides that bind to negatively charged phospholipids in bacterial membranes, causing cell damage (Mohapatra et al. 2021). Although they are commonly used in veterinary medicine, their use in humans is limited due to potential side effects (Catry et al. 2015).

Colistin resistance arises through mutations in the bacterial membranes' lipid profiles, which reduce the negative charge and hinder colistin binding (Velkov et al. 2013). Key genes involved in resistance include *crrABC*, *mgrB*, and others related to phospholipid membrane molecules (Esposito et al. 2018). Studies on *Klebsiella pneumoniae* showed that resistant strains have more L-Ara4N (Olaitan et al. 2014) in their lipid phosphate groups, decreasing colistin binding (Velkov et al. 2013).

Escherichia coli (*E. coli*), a common intestinal bacterium in humans, animals, and birds, can cause enteritis and diarrhea through virulent strains (Bélanger et al. 2011). Resistant strains of *E. coli*, often originating from contaminated food, are the main contributors to foodborne illnesses (Caine et al. 2014). The spread of resistance genes via mobile genetic elements and horizontal gene transfer (HGT) further worsens the problem (Schouten et al. 2009). The *E. coli* genome frequently includes integrons, which are known to contribute to the development of antimicrobial resistance (Solis et al. 2024). Rapid detection of antimicrobial resistance is essential due to the alarming global rise in multidrug-resistant (MDR) *E. coli* and colistin resistance. This study aims to evaluate *E. coli* resistance to colistin by detecting the *mcr-1* gene in isolates from broiler chickens using polymerase chain reaction (PCR).

Materials and Methods

Ethical statement

The present study was conducted following the ethical guidelines of the University of Agriculture, Peshawar, Pakistan. Proper ethical approval was obtained from the ethical committee responsible for lab animal care and management at the College of Veterinary Sciences, Agriculture University, Peshawar, Pakistan.

Sample collection and processing

Samples for this study were collected from three districts in Khyber Pakhtunkhwa, Pakistan: Peshawar, Charsadda, and Mardan. The samples were obtained from birds brought for postmortem examination from these districts to the poultry postmortem section of the Veterinary Research Institute in Peshawar, for disease investigation. Bird samples were collected, properly labeled with the district name, and sent to the Microbiology Lab at the College of Veterinary Sciences, University of Agriculture, Peshawar. A total of 246 sick birds showing signs and symptoms of colibacillosis were sampled, with 82 samples taken from each district. For further analysis, the samples were quickly placed in sterile Brain Heart Infusion (BHI) broth.

Isolation and biochemical identification

Samples were initially screened based on the clinical history from the owner and postmortem examination. Using an inoculating loop, fecal or cloacal samples were aseptically transferred to brain heart infusion (BHI) broth (Difco™, Becton Dickinson, Sparks, MD, USA) and incubated for 16–24 hours to promote bacterial growth. After enrichment, aliquots were streaked onto MacConkey agar plates (Difco™, Becton Dickinson, Sparks, MD, USA) supplemented with 2 µg/mL colistin sulfate and incubated at 37°C to select for colistin-tolerant colonies. Isolated colonies were then subcultured onto two types of MacConkey agar: one containing 2 mL/L colistin to enhance resistance detection and another without colistin as a control, ensuring colony purification and resistance profiling.

Bacterial isolation and identification

E. coli was identified through a series of microbiological techniques. Initially, the microorganism was cultured on MacConkey agar, a medium selective for Enterobacteriaceae, where it displayed characteristic pink colonies. These colonies, after treatment with colistin, were further analyzed on eosin methylene blue (EMB) agar. The presence of a metallic sheen on EMB agar was used as a preliminary indication of *E. coli*.

DNA extraction and gene amplification

DNA from the initially identified *E. coli* was isolated following the manufacturer's kit instructions. PCR assays targeted the *uid*A gene for identification.

Selective colonies identified as *E. coli* were obtained after overnight enrichment in BHI broth. To prepare the DNA template, 1 mL of the enriched broth culture was centrifuged at 11,000 rpm for 1 minute, and the supernatant was discarded. The pellet was then resuspended in molecular-grade water and transferred into PCR tubes. The suspension was heated in a Bio-Rad thermocycler at boiling for 20 minutes. Afterward, the lysate was centrifuged at 500 rpm for 2 minutes, and the supernatant was used as the DNA template for further molecular analysis.

Polymerase chain reaction and gene amplification

Bacterial DNA from presumptive *E. coli* isolates was extracted using boiling and Kit method for validation.

Commercial Kit: The QIAamp DNA Mini Kit (Qiagen, Germany) was used following the manufacturer's protocol.

Boiling lysis: A total of 1 mL of enriched BHI broth culture was centrifuged at 11,000 rpm for 1 minute, re-suspended in 200 µL of molecular-grade water, and heated at 95°C for 20 minutes in a Bio-Rad thermocycler. The lysate was then centrifuged at 500 rpm for 2 minutes, and the supernatant was used as a template. *E. coli* identification was confirmed through amplification of the *uidA* gene using published primers and conditions. For *mcr-1* detection, we used the primers CLR5-F (CGGTCAGTCCGTTTGTTC) and CLR5-R (CTTGGTCGGTCTGTAGGG) as previously described by Liu et al. (2016). A known *E. coli* strain (NCTC 13846), previously confirmed to harbor the *mcr-1* gene, served as a positive control, while nuclease-free water was used as a negative control. The *16S rRNA* gene was amplified as an internal control to verify reaction integrity. Cycling conditions included an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 57°C for 45 seconds, and extension at 72°C for 2 minutes. A final elongation step was performed at 72°C for 10 minutes. PCR products were separated by electrophoresis on 1% (w/v) agarose gels for 30 minutes. Visualization was carried out under ultraviolet light, and images were captured with a gel documentation system (Alpha® Imager EC, San Leandro, USA). Both DNA extraction methods produced consistent results for downstream PCR applications.

Antimicrobial susceptibility testing

To assess the antibiotic susceptibility of *E. coli* strains confirmed as colistin-resistant via PCR detection of the *mcr-1* gene, a disc diffusion test was conducted using Mueller-Hinton Agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2023). The disc diffusion method has limitations in assessing colistin susceptibility due to poor agar diffusion of polymyxins. Although our current testing followed general CLSI guidelines, we recognize that colistin-specific testing for *Enterobacterales* requires broth microdilution (BMD), and this method will be prioritized in follow-up studies to enhance accuracy.

Data analysis

The collected data were entered into Microsoft Excel and analyzed using the SPSS and Statistix 8.1 software packages for statistical evaluation.

Results

Prevalence of *E. coli* isolated from diseased poultry birds in different districts

Out of 246 samples collected from diseased poultry in three districts – Peshawar, Charsadda, and Mardan – *E. coli* was isolated in 178 cases (72.35%) using Eosin Methylene Blue agar and confirmed through molecular techniques. The district-wise distribution showed the highest *E. coli* isolation rate in Charsadda (85.37%), followed by Peshawar (69.51%) and Mardan (62.20%). Statistical analysis indicated a significant difference in *E. coli* prevalence among districts (Chi-square = 11.503, p=0.003). Additionally, molecular screening of these isolates for the *mcr-1* gene, which confers plasmid-mediated resistance to colistin, identified 34 *mcr-1*-positive strains (19.10% of the total *E. coli* isolates). Charsadda had the highest number of *mcr-1*-positive isolates (25.71%), followed by Mardan (17.65%) and Peshawar (12.28%), though the difference in *mcr-1* prevalence among districts was not statistically significant (p=0.1521), as detailed in Table 1. Figure 1 shows the transcript of the *mcr-1* genes analyzed by PCR.

Antimicrobial susceptibility profile of *mcr-1*-positive *E. coli* isolates

Although all 34 *mcr-1*-positive isolates carried the colistin resistance gene, only 26 (76.5%) exhibited phenotypic resistance or intermediate susceptibility to colistin by the disc diffusion test. Eight isolates (23.5%) remained phenotypically susceptible. This suggests a partial agreement between genotype and phenotype for colistin resistance. Table 2 shows a cumulative antibiogram of these colistin-resistant isolates. Except for tylosin resistance, the isolates displayed multidrug resistance (MDR), defined as resistance to at least three antibiotic classes. High resistance rates were seen with sulfamethoxazole (82.4%), ampicillin (64.71%), and

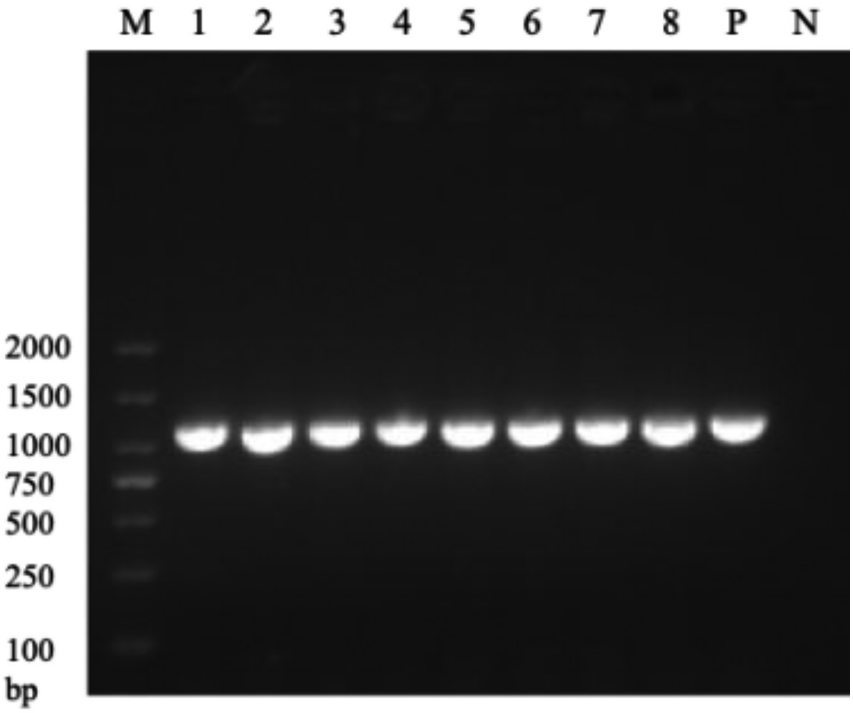

Fig. 1. Amplification of the *mcr-1* gene from colistin-resistant *Escherichia coli* isolates obtained from infected poultry. PCR products of the expected size 1165 bp are visible in lanes 1–8. Lane P: positive control (*mcr-1* gene); Lane N: negative control; Lane M: 2000 bp DNA ladder (TransGen, Beijing, China).

Table 1. Occurrence of *E. coli* and the drug-resistant *mcr-1* gene isolated from diseased poultry birds.

Districts	*E. coli*		Chi-Square	(*P*-Value)	*mcr-1*		Chi-Square	(*P*-Value)
	+ (%)	- (%)			+ (%)	- (%)		
Peshawar	57 (69.51)	25	11.503	0.003*	7 (12.28)	50	3.7668	0.1521^{ns}
Charsadda	70 (85.37)	12			18 (25.71)	52		
Mardan	51 (62.20)	31			9 (17.65)	42		
Total	178 (72.35)	68			34 (19.10)	144		

Values with p<0.05 are considered statistically significant (*), and (^{ns}) are non-significant.

Table 2. Cumulative antibiogram of colistin-resistant isolates of *E. coli* from poultry birds.

S. No	Anti-microbial Drug	Resistant (%)	Intermediate (%)	Susceptible (%)	Chi-Square
1	Tylosin	30 (88.24)^{ns}	4 (11.76)*	0 (0.00)*	
2	Ceftriaxone	15 (44.12)^{ns}	9 (26.47)^{ns}	11 (31.43)^{ns}	
3	Cefixime	13 (38.24)^{ns}	6 (17.65)^{ns}	15 (42.86)^{ns}	
4	Gentamicin	12 (35.29)^{ns}	11 (32.35)^{ns}	11 (31.43)^{ns}	
5	Cefepime	7 (20.59)*	7 (20.59)^{ns}	20 (57.14)^{ns}	
6	Ampicillin	22 (64.71)^{ns}	5 (14.71)^{ns}	7 (20.00)*	
7	Norfloxacin	22 (64.71)^{ns}	6 (17.65)^{ns}	6 (17.14)^{ns}	98.725
8	Sulfamethoxazole	28 (82.35)^{ns}	4 (11.76)*	2 (5.71)*	
9	Tetracycline	12 (35.29)^{ns}	7 (20.59)^{ns}	15 (42.86)^{ns}	
10	Meropenem	20 (58.82)^{ns}	8 (23.53)^{ns}	7 (20.00)*	
11	Ceftazidime	6 (17.65)*	6 (17.65)^{ns}	22 (62.86)^{ns}	
12	Kanamycin	20 (58.82)^{ns}	6 (17.65)^{ns}	8 (22.86)*	
13	Colistin	14 (41.18)^{ns}	12 (35.29)^{ns}	8 (22.86)*	

X-squared =, df = 24, p-value = 4.953e-11
Values with p<0.05 are considered statistically significant (*), and (ns) are non-significant.

norfloxacin (64.71%). Moderate resistance was observed for meropenem (58.82%), kanamycin (58.82%), and ceftriaxone (44.12%). Lower resistance rates were recorded for cefepime (20.59%) and ceftazidime (17.65%), with the latter being the most effective antibiotic, showing a sensitivity rate of 62.86%. The antibiogram results highlight the concerning level of co-resistance among *mcr-1*-positive isolates to multiple antibiotics.

Discussion

Antibiotic resistance is a growing concern that has a significant impact on global health and the poultry industry. Drug-resistant bacterial infections cause considerable economic losses (Sarmah et al. 2006). The routine use of antibiotics, especially for growth promotion in animals, has led to microbial adaptation, resulting in antibiotic resistance (Munday et al. 2004). In numerous countries of the world, the increasing incidence of Colistin resistance in gram-negative bacteria, especially in *E. coli,* has become a serious problem (Nguyen et al. 2015). The colistin-resistant gene, "*mcr-1*", was 1st discovered as a mobile plasmid-associated element in China. It has been widely disseminated around the world since its discovery (Liu et al. 2016). One significant factor contributing to the emergence of the *mcr-1* gene is the extensive use of colistin in the food animal industry (Trung et al. 2017). While its use in human medicine remains restricted, colistin is often employed in animal production, exacerbating the spread of resistance. Despite the universal concern, there is a lack of comprehensive examinations employing a one-health approach to directly link colistin use in animals with *mcr-1* presence in human pathogens (Koressaar et al. 2007).

The prevalence of *E. coli* was found to be 72.35% in this study in affected poultry from a selected area. This result is consistent with the observations of Hossain et al. (2008), who reported a 60% prevalence in chicken fecal samples, and Jakaria et al. (2012), whose study identified an even higher prevalence of 78.86% in cloacal specimens. However, other studies report lower prevalence rates. Rahman et al. (2004) found only a 21% prevalence in poultry, and Arslan and Eyi (2011) reported a 43% prevalence in chicken meat. These variations can be attributed to differences in sampling methods, culturing techniques, and strain variability, highlighting the need for standardized methodologies when assessing *E. coli* prevalence.

Regarding the *mcr-1* gene, our study found an overall prevalence of 19 % in diseased poultry birds. This prevalence is higher than the 13% reported by Jayol et al. (2018) and the 5% found by Yang et al. (2017) in China. Studies in Europe show even lower rates, such as 1.8% in France (Perrin-Guyomard et al. 2016) and 1.5% in Dutch supermarket chicken (Kluytmans et al. 2016). A study from Italy by Cannatelli et al. (2016) screened colistin-resistant *E. coli* isolates collected from hospitalized patients and found eight out of nine strains positive for the *mcr-1* gene. However, Nguyen et al. (2015) reported a much higher prevalence of 59% in Vietnam. These findings highlight geographical differences and suggest that antibiotic usage practices may contribute to the dissemination of the *mcr-1* gene in *E. coli*.

Our study focused particularly on detecting the *mcr-1* gene for several scientifically justified reasons. First, as the initial and most widely documented plasmid-mediated colistin resistance mechanism, *mcr-1* remains the primary variant reported in global surveillance of food animals, particularly poultry (Liu et al. 2016). Second, regional antimicrobial resistance monitoring in South Asia has consistently identified *mcr-1* as the dominant variant in livestock-associated Enterobacteriaceae. The consistent use of controls throughout our study, including *mcr-1*-positive *E. coli* NCTC 13846 as a positive control, nuclease-free water as a negative control, and 16S rRNA as an internal amplification control, ensured the reliability of these results.

Historically, polymyxin resistance, including colistin, was mainly associated with chromosomal mutations (Olaitan et al. 2014). However, the discovery of the plasmid-mediated *mcr-1* gene encoding Phosphoethanolamine transferase marked a paradigm shift in our understanding of this resistance (Liu et al. 2016). Initially detected in Enterobacteriaceae from both humans and animals by Liu et al. (2016), *mcr-1* has now achieved widespread global distribution (Webb et al. 2016). The presence of both *mcr-1* and *mcr-2* genes in Enterobacteriaceae, including *E. coli,* poses a critical threat to human health, as these genes confer resistance to one of the last-resort antibiotics (McGann et al. 2016). The continued emergence of multidrug-resistant strains underscores the need for alternative therapeutic options as the efficacy of colistin diminishes (Temkin et al. 2014).

In our study, we conducted antimicrobial susceptibility testing on 34 *mcr-1*-positive *E. coli* isolates from three districts against 13 antibiotics. Alarmingly, colistin resistance was observed in 88.24% of isolates, with complete resistance (0% susceptibility). Notably, a discrepancy was observed between the genotypic and phenotypic resistance profiles of colistin. While the *mcr-1* gene was detected in all 34 isolates, phenotypic resistance or intermediate susceptibility was evident in only

26 isolates, with the remaining 8 exhibiting susceptibility in disc diffusion assays. Such discordance has been reported previously and may result from low-level expression of the *mcr-1* gene, gene silencing, variations in promoter activity, or plasmid copy number. Furthermore, CLSI and EUCAST recommend broth microdilution (BMD) rather than disc diffusion for colistin susceptibility testing in Enterobacterales, as the latter may yield inconsistent results due to colistin's poor diffusion in agar (Poirel et al. 2017, CLSI, 2023). Future studies will incorporate BMD-based MIC determination to better assess resistance phenotypes. High resistance rates were also noted for sulphamethoxazole (82.35%), norfloxacin, and ampicillin (64.71%). However, ceftazidime showed relatively high susceptibility (62.86%) and the lowest resistance (17.65%) among the tested antibiotics. These results align with those reported in *E. coli* from poultry. Our findings are in line with a study conducted by Islam et al. (2014), who reported *E. coli* susceptibility rates of 86.67% for gentamicin, 80% for norfloxacin, and 73.33% for tetracycline, with high resistance (86.67%) to amoxicillin. The finding emphasizes the urgent need to implement stringent regulations on antibiotic use in the poultry industry. Such measures are essential to mitigate the emergence of antibiotic-resistant bacteria, which could infiltrate agricultural systems through the improper handling or application of poultry manure, thereby posing significant risks to public health and food safety (Cortés et al. 2010).

E. coli, particularly colistin-resistant strains, causes substantial economic losses in the commercial poultry industry due to increased disease prevalence, mortality, and carcass condemnation (Ewers et al. 2004). Additionally, antimicrobial resistance poses a direct threat to public health, as resistant bacteria can be transmitted through food products, including poultry meat (Johnson et al. 2007). The consumption of resistant strains of food-borne pathogens poses a significant risk of transferring resistant genes to pathogenic microorganisms in humans. This phenomenon exacerbates the global challenge of antibiotic resistance, further complicating efforts to combat this critical public health threat (Pesavento et al. 2007). The development of colistin-resistant *E. coli* in poultry highlights the important need for regulatory action to limit antibiotic usage in animal agriculture and avoid the transfer of resistance genes to the human population.

Conclusions

This study emphasizes the alarming levels of antibiotic resistance in *mcr-1*-positive *E. coli* isolates from poultry, especially against colistin (88.24%) and sulfamethoxazole (82.35%). Ampicillin and sulfamethoxazole exhibited high resistance in all districts and should be avoided, particularly in Peshawar. Conversely, ceftazidime showed better susceptibility and could be considered a viable treatment option in Peshawar, Charsadda, and Mardan, along with cefepime. These findings underscore the urgent need for continuous surveillance and the implementation of region-specific antibiotic stewardship programs to address the rise of colistin-resistant *E. coli* in poultry. The growing resistance to commonly used antibiotics highlights the importance of careful antimicrobial use in animal husbandry to safeguard both animal and human health. The phenotypic resistance data for colistin is preliminary and the genotypic detection of *mer-1* is the more relavant finding.

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References

Arslan S, Eyi A **(2011)** Antimicrobial resistance and ESBL prevalence in Escherichia coli from retail meats. J Food Safety 31: 262-267.

Bélanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM **(2011)** Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunol Med Microbiol 62: 1-10.

Caine LA, Nwodo UU, Okoh AI, Ndip RN, Green E **(2014)** Occurrence of virulence genes associated with diarrheagenic Escherichia coli isolated from raw cow's milk from two commercial dairy farms in the Eastern Cape Province, South Africa. Int J Environ Res Public Health 11: 11950-11963.

Cannatelli A, Giani T, Antonelli A, Principe L, Luzzaro F, Rossolini GM (**2016**) First detection of the *mcr-1* colistin resistance gene in Escherichia coli in Italy. Antimicrob Agents Chemother 60: 3257-3258.

Catry B, Cavaleri M, Baptiste K, Grave K, Grein K, Holm A, Jukes H, Liebana E, Navas AL, Mackay D **(2015)** Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. Int J Antimicrob Agents 46: 297-306.

Clinical and Laboratory Standards Institute (CLSI) **(2023)** Performance standards for antimicrobial susceptibility testing of anaerobic bacteria: informational supplement.

Cortés P, Blanc V, Mora A, Dahbi G, Blanco JE, Blanco M, López C, Andreu A, Navarro F, Alonso MP **(2010)** Isolation

and characterization of potentially pathogenic antimicrobial-resistant Escherichia coli strains from chicken and pig farms in Spain. Appl Environ Microbiol 76: 2799-2805.

Esposito EP, CervoniM, Bernardo M, Crivaro V, Cuccurullo S, Imperi F, Zarrilli R **(2018)** Molecular Epidemiology and Virulence Profiles of Colistin-Resistant Klebsiella pneumoniae Blood Isolates from the Hospital Agency "Ospedale dei Colli," Naples, Italy. Front Microbiol 9: 1463.

Ewers C, Janben T, Kiebling S, Philipp HC, Wieler LH **(2004)** Molecular epidemiology of avian pathogenic Escherichia coli (APEC) isolated from colisepticemia in poultry. Vet Microbiol 104: 91-101.

Hossain MT, Siddique MP, Hossain FMA, Zinnah MA, Hossain MM, Alam MK, Choudury KA **(2008)** Isolation, identification, toxin profile and antibiogram of Escherichia coli isolated from broilers and layers in Mymensingh district of Bangladesh. Bangl J Vet Med, 6: 1-5.

Islam MZ, Musekiwa A, Islam K, Ahmed S, Chowdhury S, Ahad A, Biswas PK **(2014)** Regional variation in the prevalence of *E. coli* O157 in cattle: a meta-analysis and meta-regression. PloS One 9: e93299.

Jayol A, Nordmann P, Poirel L, Dubois V **(2018)** Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing Klebsiella pneumoniae. J Antimicrob Chemo 73: 542-544.

Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, Bender J, Smith KE, Winokur PL, Belongia EA **(2007)** Antimicrobial drug – resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002–2004. Emerg Infect Dis 13: 838-846.

Kluytmans van den Bergh MF, Huizinga P, Bonten MJ, Bos M, De Bruyne K, Friedrich AW, Kluytmans JA **(2016)** Presence of MCR-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. EuroSurveill 21: 30149.

Koressaar T, Remm M **(2007)** Enhancements and modifications of primer design program Primer3. Bioinform 23: 1289-1291.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X **(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.

McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE **(2016)** Escherichia coli harboring MCR-1 and bla CTX-M on a novel IncF plasmid: first report of MCR-1 in the United States. Antimicrobial Agents Chemotherapy 60: 4420-4421.

Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, StMichael F, Cox AD **(2010)** Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. Antimicrobial Agents Chemotherapy 54: 4971-4977.

Mohapatra SS, Dwibedy SK, Padhy I **(2021)** Polymyxins, the last-resort antibiotics: Mode of action, resistance emergence, and potential solutions. J Biosci: 46: 85-103.

Munday C, Whitehead G, Todd N, Campbell M, Hawkey P **(2004)** Predominance and genetic diversity of community and hospital-acquired CTX-M extended-spectrum β-lactamases in York, UK. J Antimicrob Chemother 54: 628-633.

Nguyen VT, Carrique-Mas JJ, Ngo TH, Ho HM, Ha TT, Campbell JI, Nguyen TN, Hoang NN, Pham VM, Wagenaar JA **(2015)** Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam. J Antimicrob Chemother 70: 2144-2152.

Olaitan AO, Morand S, Rolain J-M **(2014)**. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbio 5: 643-661.

Perrin-Guyomard A, Bruneau M, Houée P, Deleurme K, Legrandois P, Poirier C, Sanders P **(2016)** Prevalence of *mcr-1* in commensal Escherichia coli from French livestock, 2007 to 2014. EuroSurveill 21: 30135.

Pesavento G, Ducci, B, Comodo N, L Nostro A **(2007)** Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). Food Control 18: 196-200.

Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S **(2018)** Antimicrobial Resistance in Escherichia coli. Microbiol Spect 6:1-27.

Rahman MA, Samad MA, Rahman MB, Kabir SM **(2004)** Bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in natural and experimental infections in chickens. Bangl J Vet Med 2: 1-8.

Sarmah AK, Meyer MT, Boxall AB **(2006)** A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65: 725-759.

Schouten J, Graat E, Frankena K, Van-Zijderveld F, De-Jong M **(2009)** Transmission and quantification of verocytotoxin-producing Escherichia coli O157 in dairy cattle and calves. Epidemiol Infect 137: 114-123.

Solis MN, Loaiza K, Torres Elizalde L, Mina I, Šefcová MA, Larrea Álvarez M **(2024)** Detecting Class 1 Integrons and Their Variable Regions in Escherichia Coli Whole-Genome Sequences Reported from Andean Community Countries. Antibiotics (Basel) 13: 394-408.

Temkin E, Adler A, Lerner A, Carmeli Y **(2014)** Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. Ann N Y Acad Sci 1323: 22-42.

Trung NV, Matamoros S, Carrique-Mas JJ, Nghia NH, Nhung NT, Chieu T T, Mai HH, vanRooijen W, Campbell J, Wagenaar JA, Hardon A, Mai NT, Hieu TQ, Thwaites G, de-Jong MD, Schultsz C, Hoa NT **(2017)** Zoonotic Transmission of MCR-1 Colistin Resistance Gene from Small-Scale Poultry Farms, Vietnam. Emerg Infect Dis 23: 529-532.

Velkov T, Soon RL, Chong PL, Huang JX, Cooper MA, Azad MA, Baker MA, Thompson PE, Roberts K, Nation RL **(2013)** Molecular basis for the increased polymyxin susceptibility of Klebsiella pneumoniae strains with under-acylated lipid A. Innate Immun 19: 265-277.

Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, Bugarel M, Ison SA, Scott HM, Loneragan GH **(2016)** Dissemination of the MCR-1 colistin resistance gene. Lancet Infect Dis 16: 144-145.

Yang SC, Lin CH, Aljuffali IA, Fang JY **(2017)** Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. Archives of Micro 199: 811-825.