

First report of *bla_{OXA}* and *mcr-2* genes co-existing in a multidrug-resistant *Pseudomonas aeruginosa* isolate recovered from a layer chicken flock in Türkiye

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic and versatile pathogen that causes infections in both humans and animals. It is one of the ESKAPE pathogens, characterized by the ability to rapidly acquire resistance to antimicrobials. In recent years, multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) *P. aeruginosa* have been reported worldwide in humans and chickens. In poultry, *P. aeruginosa* is frequently isolated and spreads easily due to its multiple transmission routes and its ability to infect birds of all ages. As a zoonotic pathogen, it poses a risk to humans via the consumption of contaminated poultry products. This study aimed to isolate *P. aeruginosa* from a layer chicken flock in Türkiye and investigate its antimicrobial resistance (AMR) and biofilm formation ability. Isolation and identification were performed by conventional bacteriological analysis and sequence analysis of the 16S *rRNA* gene. Following phenotypic AMR testing, biofilm formation was quantitatively analyzed, and eleven AMR genes were screened by PCR. The recovered *P. aeruginosa* isolate exhibited resistance to aztreonam, colistin, and meropenem, thereby meeting the criteria for MDR. It also tested positive for *bla_{OXA}* and *mcr-2* resistance genes and was defined as a weak biofilm producer. To the best of our knowledge, this is the first report of *bla_{OXA}* and *mcr-2* genes co-existing in an MDR *P. aeruginosa* isolate recovered from a layer chicken flock in Türkiye. These findings highlight the potential risk posed by MDR and biofilm forming *P. aeruginosa* in poultry environments to both human and animal health.

Keywords: Carbapenem, Colistin, Layer chicken, *mcr-2*, *P. aeruginosa*

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Introduction

Pseudomonas species are opportunistic and versatile bacteria, capable of causing a wide range of infections in both humans and animals. Among them, *Pseudomonas aeruginosa* (*P. aeruginosa*) is considered the most frequent nosocomial pathogen (22). It is also the most common *Pseudomonas* species found in poultry worldwide (2). In poultry, *P. aeruginosa* is associated with septicemia, sinusitis, keratoconjunctivitis, perihepatitis, pericarditis, respiratory infections, and embryonic deaths (36). It is frequently isolated and spreads rapidly due to its multiple transmission routes and its ability to infect birds of all ages (32). For example, Elghazaly et al. (12),

Algammal et al. (2), and Eidaroos et al. (10) reported *P. aeruginosa* isolation from chickens at notable prevalences ($\geq 25.0\%$).

P. aeruginosa is one of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp.), characterized by the ability to rapidly acquire resistance to antimicrobial agents. Consequently, infections caused by ESKAPE pathogens are difficult to treat and often associated with limited therapeutic options (28). This creates a major challenge in managing antimicrobial resistant *P. aeruginosa* infections, particularly in developing

countries (26). The emergence and spread of multidrug-resistant (MDR, non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial classes), extensively drug-resistant (XDR, non-susceptible to ≥ 1 agent in all but ≤ 2 antimicrobial classes), and pandrug-resistant (PDR, non-susceptible to all antimicrobial agents) *P. aeruginosa* isolates have been linked to increased mortality, treatment failures, and therapeutic challenges (1). In recent years, MDR, XDR, and PDR *P. aeruginosa* have been reported worldwide in both humans and chickens (2, 3, 10, 14, 15, 18). For example, Eid et al. (9) reported that all *P. aeruginosa* isolated from broilers in Egypt were XDR, while XDR *P. aeruginosa* were also detected in layers. For example, Eidaroos et al. (10) reported that 95.0% of *P. aeruginosa* isolates from layers in Egypt were XDR. The present study aimed to isolate *P. aeruginosa* from a layer chicken flock in Türkiye and investigate its antimicrobial resistance (AMR) and biofilm formation ability.

Case History

The study material was a 25 g litter sample, collected from a commercial layer chicken flock in Türkiye in 2025. For tenfold dilution, the sample was suspended in buffered peptone water (BPW; Oxoid, UK) A loopful of the enriched sample was streaked onto 5% blood agar (Oxoid,

UK) and nutrient agar (Oxoid, UK). Suspected colonies were subcultured onto cystine lactose electrolyte-deficient (CLED; BD, France), MacConkey (Oxoid, UK), triple sugar iron (TSI; Oxoid, UK), and xylose lysine deoxycholate (XLD; Oxoid, UK) agar plates. The incubations were made at 37°C overnight. After Gram staining, biochemical identification was carried out using catalase, oxidase, oxidation-fermentation, growth at 42°C, nitrate reduction, citrate utilization, indole, motility, and urease tests (21).

Sequence analysis of the 16S *rRNA* gene was performed to confirm the biochemical identification of the isolate. The 16S *rRNA* gene was amplified with universal 27F and 1492R primers (26). Amplification products were sequenced using the BigDye Direct Cycle Sequencing kit (Applied Biosystems, USA) on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA). The consensus sequence was compared with 16S *rRNA* reference sequences in the GenBank database using the Basic Local Alignment Search Tool (BLASTn) and was deposited there under the assigned accession number.

The AMR of the *P. aeruginosa* isolate was examined using both phenotypic and genotypic tests. Twelve antimicrobials from six classes were tested by the Kirby-Bauer disc diffusion method, whereas colistin resistance was tested by the broth dilution method (Table 1).

Table 1. Antimicrobials and specific primers used in the antimicrobial resistance tests.

Antimicrobial class	Antimicrobial	Resistance gene	Sequence (5' to 3')	Size (bp)	Tm (°C)	Reference
Aminoglycosides	Amikacin (30 µg)	<i>aac(6')-Ib</i>	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482	55	(25)
	Gentamicin (10 µg) Netilmicin (30 µg) Tobramycin (10 µg)	<i>aaC(3)-IIa</i>	F: ACTGTGATGGGATACGCGTC R: CTCGCTCAGCGTTTCAGCTA	482	60	(34)
Monobactams	Aztreonam (30 µg)	-	-	-	-	-
Cephems	Cefepime (30 µg)	<i>bla_{CTX-M}</i>	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	52	(4)
	Ceftazidime (30 µg)	<i>bla_{SHV}</i>	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTGCTCGG	795	50	(37)
		<i>bla_{TEM}</i>	F: GCACGAGTGGGTTACATCGA R: GGTCTCTCCGATCGTTGTCAG	310	60	(5)
Beta-lactamase inhibitor comb.	Piperacillin-tazobactam (100/10 µg)	-	-	-	-	-
Carbapenems	Imipenem (30 µg)	<i>bla_{NDM}</i>	F: GCAGCTTGTCGGCCATGCGGGC R: GGTGCGCAAGCTGAGCACCGCAT	782	52	(8)
	Meropenem (30 µg)	<i>bla_{OXA}</i>	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438	55	(34)
Fluoroquinolones	Ciprofloxacin (5 µg)	<i>qnrA</i>	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTCAGGTCA	516	53	(30)
	Ofloxacin (5 µg)	<i>qnrB</i>	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	53	(29)
Polymyxins	Colistin	<i>mcr-1</i>	F: AGTCCGTTTGTCTTGTGGC R: AGATCCTTGGTCTCGGCTTG	320	58	(27)
		<i>mcr-2</i>	F: CAAGTGTGTTGGTCGCAGTT R: TCTAGCCCCACAAGCATACC	715	58	(27)

Findings were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints for *P. aeruginosa* (7). *E. coli* ATCC 25922 was used as the quality control. MDR was defined according to existing criteria (20). The multiple antibiotic resistance (MAR) index was calculated as previously described (17). The value >0.2 was considered as indicating high risk contamination (2). The presence of eleven resistance genes was screened by PCR (Table 1).

Biofilm formation was quantitatively analyzed using the microtiter plate method (33). Optical densities (ODs) were measured at 595 nm using a microplate reader (BioTek, USA). *E. coli* ATCC 25922 was used as the positive control while sterile TSB (200 μ L) was used as the negative control to determine the cut-off OD (OD_c). The isolate was classified based on its biofilm-forming ability: non-biofilm producer ($OD \leq OD_c$), weak biofilm producer ($OD_c < OD \leq 2OD_c$), moderate biofilm producer ($2OD_c < OD \leq 4OD_c$), or strong biofilm producer ($4OD_c < OD$).

Results

The recovered isolate exhibited hemolysis on blood agar and displayed blue-green pigmentation with a characteristic grape-like odour on nutrient agar at both 37°C and 42°C. It was negative for hydrogen sulfide production on XLD agar. On MacConkey and CLED agar plates, it grew as pale-large and green-large colonies, respectively (Figure 1). Microscopic examination confirmed Gram-negative rods. Biochemical testing revealed that the isolate was positive for catalase, oxidase, citrate utilization, nitrate reduction, motility, but negative for urease and indole. It showed an alkaline reaction with no change in TSI agar and an oxidative pattern in the OF medium. Based on these characteristics, the isolate was identified as *P. aeruginosa*. This identification was further confirmed by sequence analysis of the 16S *rRNA* gene using the BLASTn tool. The sequence was deposited in the GenBank database under accession number PV983597. The sequence is similar to the partial 16S *rRNA* gene sequences from around the world. Comparative analysis indicated a 99.71% similarity with KX879787, KX879794, and KX879799 (China) and a

99.56% similarity with KY285994, OR077732, MF144545, MG009247, and LC508781 (Pakistan, India, Peru, South Korea, and Japan, respectively).

P. aeruginosa isolate was resistant to aztreonam, meropenem, and colistin. It was considered to be MDR. Among the eleven resistance genes screened, the isolate was positive for *bla*_{OXA} and *mcr*-2, consistent with the phenotypic AMR findings. The MAR index was 0.23, indicating a risk of antibiotic contamination. Regarding the biofilm formation analysis, the mean OD of the negative control (OD_c) was 0.058, whereas that of the isolate was 0.099, classifying the isolate as a weak biofilm producer.

Discussion and Conclusion

P. aeruginosa is an opportunistic pathogen of poultry. It can easily spread among flocks and cause death across a wide age range. As a zoonotic pathogen, it can cause infections in humans through consumption of contaminated poultry products (31). Therefore, it is important to detect *P. aeruginosa* in the poultry environment for assessing the potential risk to public health. Accordingly, this study aimed to isolate *P. aeruginosa* from a layer chicken flock in Türkiye and investigate its antimicrobial resistance and biofilm formation ability.

In this study, the *P. aeruginosa* isolate was defined as MDR. These findings are consistent with previous reports, which have highlighted resistant *P. aeruginosa* as a critical concern in chickens (2, 10). Eid et al. (9) reported a 100.0% prevalence of XDR in broiler-derived *P. aeruginosa* isolates. They concluded that eliminating *P. aeruginosa* from chicken flocks will be a major challenge. Algammal et al. (2) reported that 40.7% of *P. aeruginosa* isolates obtained from broilers were XDR. Comparable findings have also been reported in layer chickens. Eidaroos et al. (10) reported that 95.0% of *P. aeruginosa* isolates from layers were XDR, while Morsy et al. (23) reported 100.0% prevalence of MDR *P. aeruginosa* isolates from broiler and layer chickens. The overuse and misuse of antimicrobials in the poultry industry, coupled with *P. aeruginosa*'s ability to acquire AMR genes from other superbugs triggers, have contributed to the

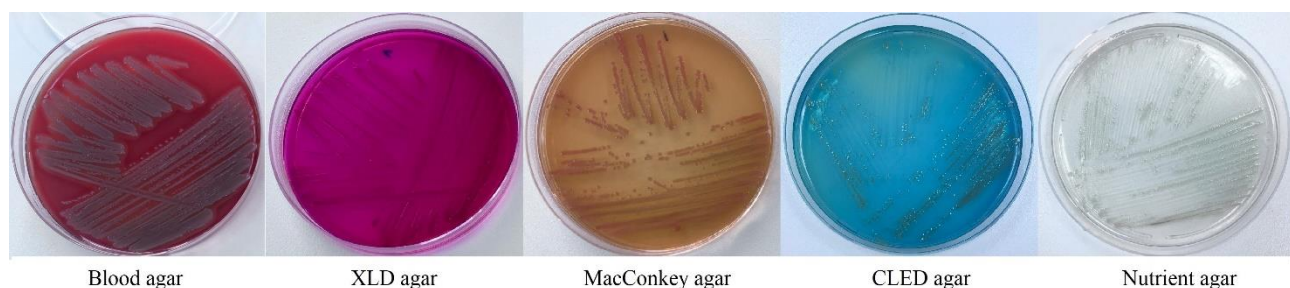


Figure 1. *P. aeruginosa* colonies on different media for cultural morphological identification.

increasing prevalence of MDR and XDR *P. aeruginosa* isolates (2).

Regarding antibiotic contamination, the MAR index for the recovered isolate was 0.23, which indicates a risk of antibiotic contamination caused by the recovered isolate. However, it should be noted that this study is a report based on a single *P. aeruginosa* isolate. With a larger number of isolates, higher MAR indices could potentially be detected across a broader range. Previous studies have reported higher MAR indices for *P. aeruginosa* isolated from chickens. Badr et al. (3), Eid et al. (9), and Algammal et al. (2) reported MAR indices ≥ 0.4 for *P. aeruginosa* isolates recovered from broilers. Similarly, Eidaroos et al. (10) reported MAR indices ranging from 0.39 to 0.83 for *P. aeruginosa* isolates from layer chickens. These high MAR values may indicate misuse of antibiotics in the poultry industry (28).

In the present study, the isolate was resistant to aztreonam, meropenem, and colistin, thereby meeting the criteria for MDR. Moreover, the isolate was positive for *bla_{OXA}* and *mcr-2*, the genes are responsible for resistance to carbapenem and colistin, respectively. To the best of our knowledge, this study represents the first report of *bla_{OXA}* and *mcr-2* resistance genes co-existing in an MDR *P. aeruginosa* isolate recovered from a layer chicken flock in Türkiye. Carbapenems are frequently used to treat *P. aeruginosa* infections because of their stability against most beta-lactamases (14, 18). However, in 2017, carbapenem-resistant *P. aeruginosa* was added to the WHO priority list of AMR bacteria (35). Previously, Mohamed et al. (22) reported that 71.4% of chicken-originated *P. aeruginosa* isolates were resistant to meropenem, while Ejikeugwu et al. (11) found a resistance rate of 60.5% in poultry-originated isolates. Meanwhile, Algammal et al. (2), Ramatla et al. (26), and Salem et al. (31) detected broiler-originated *P. aeruginosa* isolates that were positive for the *bla_{OXA}* gene. Colistin is frequently used as a last-resort antimicrobial for the treatment of MDR Gram-negative bacterial infections (19). Gill et al. (13) showed that colistin is a significant option for infections caused by MDR *P. aeruginosa*. However, the increasing prevalence of infections associated with MDR, XDR, and carbapenem-resistant *P. aeruginosa* isolates has led to the extensive use of colistin, thereby helping to increase colistin resistance rates (24). Rizk et al. (28) reported that 12 out of 38 *P. aeruginosa* isolates from broiler flocks were resistant to colistin. Badr et al. (3) and Algammal et al. (2) reported resistance rates to colistin sulfate in broiler-originated *P. aeruginosa* AMR isolates of 65.6% and 18.5%, respectively. Colistin resistance is encoded by plasmid-mediated *mcr* genes, from *mcr-1* to *mcr-10* (16). The horizontal and vertical transfer of these genes has accelerated the dissemination

of colistin-resistant isolates (24). Salem et al. (31) and Ramatla et al. (26) detected that 18.2% and 21.7%, respectively, of broiler-derived *P. aeruginosa* isolates carried *mcr-1*.

Biofilm formation is a key virulence factor of *P. aeruginosa*, enabling the bacterium to persist and survive under varied environmental conditions by colonizing different surfaces (6). It is considered one of the main mechanisms contributing to AMR in pathogens (22). In our study, the *P. aeruginosa* isolate exhibited weak biofilm-forming ability. Previous studies have reported that 100.0% of chicken-originated *P. aeruginosa* isolates were capable of forming biofilms (6, 22, 36). Similarly, Eidaroos et al. (10) found that most layer-chicken-originated *P. aeruginosa* isolates formed biofilms. These findings suggest that the presence of biofilm-forming pathogens should be considered when implementing sanitation and biosecurity procedures in chicken flocks.

In conclusion, to the best of our knowledge, this is the first report of *bla_{OXA}* and *mcr-2* resistance genes co-existing in a biofilm-forming MDR *P. aeruginosa* isolate recovered from a layer chicken flock in Türkiye. These findings highlight the potential risk to both human and animal health posed by biofilm-forming MDR *P. aeruginosa* in poultry environments.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

SSI and MA conceived the idea and designed the report. The authors have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant feedback and helped shape the report.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative Artificial Intelligence

The authors declare that generative artificial intelligence (AI) and AI-assisted technologies were not used in the writing of this manuscript or in the preparation of figures.

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