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Isolation and molecular identification of multidrug-resistant Pseudomonas aeruginosa isolated from broiler chickens in Fayoum, Egypt

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Article information	Abstract
<i>Article history:</i> Received 08 May, 2024 Accepted 10 July, 2024 Published online 23 September, 2024	In the poultry industry, the Gram-negative bacterium Pseudomonas aeruginosa is gaining importance as an emerging opportunistic pathogen with notable clinical implications. This Gram-negative pathogen can contaminate hatcheries, resulting in a range of severe respiratory symptoms, enteritis, septicaemia, keratitis, sinusitis, omphalitis,
<i>Keywords</i> : <i>P. aeruginosa</i> Broiler Chickens Antimicrobials Resistance	nephritis, and rapid morbidity and mortality, which indicates the diverse pathogenic potential of this pathogen within avian populations. This study collected 480 samples (120 each) from liver, lung, gallbladder, and kidney of broiler chickens of different ages and examined bacteriologically. The overall isolation rates of P. aeruginosa were ranged from 70.8 to 83.3%. Phenotypically, the antibiogram of the selected isolates (n=30) revealed that 96.66% were resistant to three or more antibiotics from different antimicrobial groups, thus
<i>Correspondence:</i> M.E. Elkhayat <u>manar.elkhayat@fvtm.bu.edu.eg</u>	indicating multidrug resistant to three of hiore antibiotics from different antimicrobial groups, thus indicating multidrug resistance (MRD), of which the highest resistance was to amoxicillin 100%, piperacillin 96.66%, gentamycin 86.66%, ofloxacin 80%, cefepime 63.33%, ceftazidime63.33%, levofloxacin 53.3% and ciprofloxacin 53.3% followed by apramycin 36.66% and doxycycline 36.66. In comparison, 66.6% of the isolates were sensitive to the amikacin. Polymerase chain reaction (PCR) was used for determining five resistance genes in ten selected MDR P. aeruginosa isolates. The result revealed that 100% of the tested isolates harbored the <i>mexR</i> and <i>ampC</i> resistance genes. Furthermore, the prevalence of <i>blaOXA</i> , <i>ermB</i> , and <i>arr</i> genes were 90, 80, and 50%, respectively.

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Introduction

P. aeruginosa poses a severe threat to the poultry industry as it influences birds of all ages and causes noteworthy economic losses to the poultry industry. This Gram-negative, motile, non-spore-forming bacillus (1-3) can contaminate hatcheries, causing severe respiratory symptoms, enteritis, septicemia, keratitis, sinusitis, omphalitis, nephritis, and rapid morbidity and mortality, demonstrating its diverse pathogenic potential within avian populations (4-7).Infection is facilitated by poor hygiene practices and various routes of contamination, such as skin wounds, tainted vaccinations, egg dipping or inoculation, and contaminated injection needles (8). The emergence of antimicrobial resistance among P. aeruginosa strains is an urgent global health concern, especially in developing countries, where indiscriminate antibiotic use is promoting the spread of multidrug-resistant strains (9). P. aeruginosa exhibits high adaptability to genetic changes, leading to multidrug resistance, especially when subjected to indiscriminate treatments (10). Notably, antibiotic resistance including has been reported, aminoglycosides,

monobactams, carbapenems, third-generation cephalosporins, and aminopenicillins (11,12). Moreover, P. aeruginosa employs both intrinsic and acquired mechanisms to resist antibiotics, including restricted outer membrane permeability, synthesis of antibiotic-inactivating enzymes, e.g., β -lactamases, and efflux systems to expel antibiotics (13). Of particular concern are β -lactamases, particularly class C cephalosporinases (ampC- β -lactamases), which confer resistance to cephalosporins, penicillin, and β lactamase inhibitors (14). These mechanisms underscore the challenge of controlling P. aeruginosa infections in clinical practices. Recent studies have emphasized the prevalence of multidrug-resistant P. aeruginosa strains in poultry, giving rise to an alarming challenge to animal and public health. These strains exhibit resistance to many antibiotics, necessitating the development of alternative strategies for their control (15). Understanding the mechanisms underlying P. aeruginosa resistance is essential for effective management and treatment. These mechanisms include changes in genetic structure that render conventional antibiotic therapy ineffective against various antibiotics. To investigate the presence of multidrug-resistant microorganisms of public health concern, PCR screening for the most common antibiotic resistance genesis is needed (16). The blaOXA gene of P. aeruginosa isolates is one of the major families of ESBLs classified into molecular class D and functional group 2d (oxacillinase-like carbapenemases) and is associated with resistance to oxacillicins, cephalosporins, and carbapenems (17). One of the most distinctive features of antibiotic resistance is the efflux pump system that pumps antibiotics out of cells. For example, the *mexR* gene encodes a transcriptional repressor of the mexAB-oprM efflux pump that effluxes aztreonam (18). The ermB gene encodes a methyltransferase that causes ribosomal methylation, reducing bacterial susceptibility and conferring resistance to macrolides, lincosamides, and streptogramins (19). Moreover, P. aeruginosa often develops resistance to antibiotic treatment through biofilm formation. P. aeruginosa contains an integron-encoded ribosyl transferase called the aminoglycoside regulatory response (arr) gene responsible for biofilm production (20).

This study investigates the resistance profile and molecular mechanisms underlying antibiotic resistance of *P. aeruginosa* isolated from broiler chickens in Fayoum governorate, Egypt. This will contribute to targeted strategies to halt the spread of multidrug-resistant *P. aeruginosa* and protect animal welfare and public health.

Materials and methods

Ethical approval

The animal use protocol in this study was approved by the Ethical Approval Committee of the Faculty of Veterinary Medicine, Benha University, Egypt, under the Ethical approval number (BUFVTM08-03-24).

Sampling

A total of one hundred twenty broiler chickens (n=120) of different ages were obtained from various farms in El-Fayoum Governorates. The samples included 30 one-day-old chicks, 70 birds aged 3-5 weeks, and 20 healthy birds. Four hundred eighty samples were harvested from internal organs (liver, lung, gallbladder, kidney), including 120 samples from each organ. Samples were collected from diseased, freshly dead, and healthy chickens. Samples were transported in ice boxes and then submitted for bacteriological examination.

Isolation and biochemical identification of *P.aeruginosa*

Each bird's liver, lung, gallbladder, and kidney samples were individually cultured in nutritional broth (Oxoid) and incubated for 24h at 37°C for primary enrichment. A loopful of broth was spread on Pseudomonas Cetrimideagar (Oxoid) and followed by incubation under aerobic conditions at 37°C for 24h (21). The isolates were presumptively identified as *P. aeruginosa* based on cultural characteristics and biochemical tests. Furthermore, *P. aeruginosa* could be determined by its characteristic production of the blue-green pigment pyocyanin and its characteristic grape-like odor, and its colonies are mostly oxidase-positive (21-23).

Bacterial preservation

Single colonies with characteristic colonial appearance and morphological features of *P. aeruginosa* were selected and inoculated into a 0.5% semisolid agar medium. The agar was then incubated at 37°C for 24 hours and kept at a temperature of 4°C.In addition, a 20% bacterial glycerol stock was prepared and stored at -20°C (21).

In-vitro antibiotic susceptibility testing of *P. aeruginosa* isolates

Thirty isolates (n=30) were selected for antimicrobial susceptibility testing using the disk diffusion technique (20 isolates from diseased birds and 10 from apparently healthy birds). Suspensions of isolates were prepared according to McFarland Turbidity Standard Tube No. 0.5 (equivalent to approximately 1.5 x 108 CFU/ml) and inoculated on Mueller-Hinton agar plates (Oxoid). Twelve antibacterial discs (Oxoid) including Amikacin 30µg/disk, Gentamycin 10µg/disk, Apramycin 30µg/disk, Cefotaxime 30µg/disk, Cefepime 30µg/disk, Ceftazidime 30µg/disk, Doxycycline 30µg/disk, Ciprofloxacin 5µg/disk, Levofloxacin 5µg/disk, Ofloxacin 5µg/disk, Amoxicillin 10µg/disk and Piperacillin 100µg/disk were used and then incubated at 37°C for 24h. Zones of inhibition were then measured and interpreted according to Clinical and Laboratory Standard Institute guidelines (24).

Molecular identification of *P. aeruginosa* antimicrobialresistant genes

The molecular identification of antimicrobial-resistant genes in *P. aeruginosa* was conducted using polymerase chain reaction (PCR) targeting five resistance genes: *blaOXA*, *ermB*, *arr*, *mexR*, and *ampC*. Ten representative *P. aeruginosa* strains (n=10) were selected for genotypic resistance screening. Genomic DNA from confirmed cultures was extracted using the QIAamp DNA Extraction Miniprep Kit according to the manufacturer's instructions. The primer sequences and sizes of the amplified products are shown in table1. The PCR amplification was done in a 25 µl reaction mixture consisting of 12.5 μ l Emerald Amp GT PCR master mix (Takara, Code No. RR310A), 1 μ l each of forward and reverse primers, 5.5 μ l of Nuclease-free molecular biology grade water, and 5 μ l of test DNA. The thermal profile involved a primary denaturation step at 94°C for 5 minutes, followed by 35 cycles of secondary denaturation at 94°C for 30 seconds, annealing at 55°C for 40 seconds (for *arr* and *mexR* genes), 50°C for 40 seconds (for *ampC* and *ermA* genes), or 54°C for 60 seconds. This was followed by a final extension step at 72°C for 10 minutes, and the reaction was then held at 4°C until stopped.

Table1: Oligonucleotide primers sequences

Primer		Sequence	Product size (bp)	Reference
arr	Forward	AGCGCATCACCCCAGCAAC	685	(25)
	Reverse	CGCCAAGTGCGAGCCACTGA		
mexR	Forward	GCGCCATGGCCCATATTCAG	637	(26)
	Reverse	GGCATTCGCCAGTAAGCGG		
ampC	Forward	TTCTATCAAMACTGGCARCC	550	(27)
	Reverse	CCYTTTTATGTACCCAYGA		
blaOXA-1	Forward	ATATCTCTACTGTTGCATCTCC	619	(28)
	Reverse	AAACCCTTCAAACCATCC		
ermB	Forward	GAAAAAGTACTCAACCAAATA	639	(29)
	Reverse	AATTTAAGTACCGTTACT		

Results

Prevalence of P. aeruginosa isolates in broiler chickens

The results showed a substantially high isolation rate of *P. aeruginosa* from the 480 internal organ samples collected from broiler chickens (n=120) regardless of age and health status, with percentages ranging from 70.8% to 83.3% (Figure 1a). Analysis of the site of isolation revealed varying prevalence rates of *P. aeruginosa* across different organs, with the highest prevalence observed in the gall bladder 100/120 (83.3%), followed by the lung 97/120 (80.8%), kidneys 87/120 (72.5%) and liver 85/120 (70.8%), (Figure 1b).

Prevalence of *P. aeruginosa* isolates in broiler chickens of different age groups

In addition, the isolation rates of *P. aeruginosa* from specific internal organs of broiler chickens of different age groups were studied. The results revealed that for the one-day-old chicks (n=30), the highest prevalence was detected in the gall bladder, with 100% of samples (n=30) yielding Pseudomonas isolates, followed by the lung 27/30 (90%), liver 24/30 (80%), and kidneys 21/30 (70%). Among 3-5-week-old broilers (n=70), the highest isolation rate was observed in the lung 60/70 (85.7%), followed closely by the kidneys 59/70 (84.2%), liver 58/70 (82.8%), and gall bladder 55/70 (78.5%). Notably, in healthy birds (n=20), the gall

bladder exhibited the highest isolation rate 15/20 (75%), followed by the lung 10/20 (50%), kidneys 7/20 (35%), and liver 3/20 (15%) (Figure 1c).

Antimicrobial susceptibility profile of *P. aeruginosa* isolates

The antimicrobial susceptibility profile of P. aeruginosa isolates was investigated. A total of 30 P. aeruginosa isolates, including 20 from diseased birds and 10 from apparently healthy ones, were tested. Strikingly, all tested strains exhibited 100% resistance to amoxicillin and cefotaxime, with similarly high levels of resistance observed towards Piperacillin (96.66%), gentamycin (86.66%), and ofloxacin (80%). Varying degrees of resistance were also recorded against cefepime and ceftazidime (63.33% for each) and levofloxacin and ciprofloxacin (53.3% for each). In contrast, lower resistance levels were observed towards apramycin and doxycycline (36.66% for each). Notably, the isolates displayed the highest sensitivity to amikacin (66.6%). Remarkably, 29 isolates (96.66%) exhibited resistance to more than three antibiotic agents across different antimicrobial classes, indicative of multi-drug resistance (MDR). Additionally, isolates recovered from diseased birds demonstrated notably higher degrees of resistance and lower susceptibility to most antibiotics compared to those recovered from apparently healthy birds, except for amoxicillin and cefotaxime where both P. *aeruginosa* isolated from diseased and healthy birds showed 100% resistance, in addition to apramycin, ofloxacin and ceftazidime, where *P. aeruginosa* from apparently healthy birds showed higher resistance compared to *P. aeruginosa* isolated from diseased broiler chickens (Figure 2).

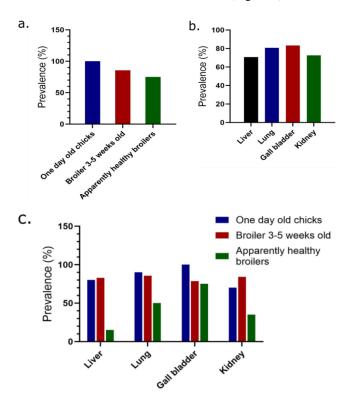


Figure1: Prevalence of *P. aeruginosa* in broiler chicken: a. Prevalence of *P. aeruginosa* in one-day-old chicks (n=30), Broilers 3-5 weeks old (n=70) and healthy broilers (n=20) collected from Fayoum Governorate. b. The total prevalence of *P. aeruginosa* in different internal organs harvested from the total broiler chicken examined (n=120). c. Prevalence of *P. aeruginosa* in different internal organs harvested from one-day-old chicks (n=30), Broilers 3-5 weeks old (n=70), and healthy broilers (n=20).

Results of occurrence of targeted resistance genes among *P. aeruginosa* isolates

The results for PCR amplification of some resistance genes in ten multidrug-resistant *P. aeruginosa* isolates showed that all examined isolates were positive for*mexR* and *ampC* resistance genes with a PCR product size in a percentage of 100% per each gene. The incidence rates for the *blaOXA*, *ermB*, and *arr* genes were 90%, 80%, and 50%, respectively. Furthermore, the results revealed that 3 of the 10 isolates were PCR positive for the 5 resistance genes examined (Figure 3).

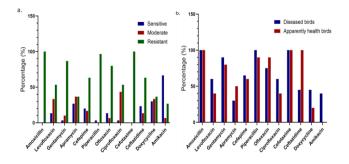


Figure 2: a. antimicrobial resistance profile of *P. aeruginosa* isolated from broiler chicken: b. antimicrobial resistance profile of *P. aeruginosa* isolated from diseased broiler chicken vs. *P. aeruginosa* isolated from apparently healthy broiler chicken.

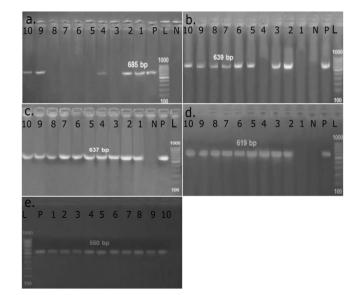


Figure 3: Prevalence of some resistance genes among the examined MDR P. aeruginosa isolates Lanes 1-10: tested DNA, L; 100-1000 bp DNA ladder, P: Positive control, N: Negative control: a. Prevalence of arr resistance gene among the examined MDR P. aeruginosa isolates with PCR amplification product of 685 bp b. Prevalence of ermB resistance gene among the examined MDR P. aeruginosa isolates with PCR amplification product of 639 bpc. Prevalence of mexR resistance gene among the examined MDR P. aeruginosa isolates with PCR amplification product of 637 bpd. Prevalence of *blaOXA-1* resistance gene among the examined MDR P. aeruginosa isolates with PCR amplification product of 619 bpe. Prevalence of ampCresistance gene among the examined MDR P. aeruginosa isolates with PCR amplification product of 550 bp.

Discussion

The presence of P. aeruginosa in poultry is of great concern due to the associated economic losses to the poultry industry and its ability to induce severe respiratory infections in humans. P. aeruginosa has been consistently isolated from various poultry sources, including chicken meat and oral and cloacal swabs (6,30). Notably, Pseudomonas infections have been linked to substantial financial burdens in chicken farms, with confirmed fatalities and sequelae including sinusitis, keratitis, respiratory symptoms, and septicemia (31). Hence, prompt isolation and identification of P. aeruginosa is essential for effective control measures. In this study, we conducted bacteriological and molecular studies on samples obtained from the liver, lung, gall bladder, and kidneys (120 each) from broiler chickens of different ages to investigate further the prevalence and antimicrobial resistance profile of P. aeruginosa isolates.

Our findings demonstrated high isolation rates of P. aeruginosa across various internal organs in broiler chickens regardless of age group and health status, with percentages ranging from 70.8% to 83.3%. The gall bladder exhibited the highest isolation rate among one-day-old chicks and healthy broilers, highlighting the potential reservoir for pathogen dissemination. These findings highlight the widespread prevalence of P. aeruginosa in broiler chickens and emphasize the importance of continued surveillance and control measures to minimize the related hazards to animal and human health. Our findings of relatively high prevalence rates of P. aeruginosa in broiler chickens contradict those reported by previous studies. For instance, Badr et al. (32) identified thirteen isolates of P. aeruginosa from diseased chickens, whereas Elsayed et al. (2) reported a lower infection rate of 22.9% among broiler chickens.

Similarly, Abd El-Hafeez*et al.* (33) investigated the frequency of *P. aeruginosa* in broiler chicken kidneys and reported an infection rate of 10.4%. Ohore *et al.* (34) reported a prevalence of 28.3% in poultry samples. These discrepancies in isolation rates could be due to changes in pathogenicity, virulence factors, disease severity, the host's immunological status, geographical locations, or environmental factors influencing bacterial colonization.

In recent years, the increasing use of antimicrobials in animal husbandry has significantly contributed to the global burden of antimicrobial resistance (35). The intensification of farming practices, particularly in developing countries, has increased the use of antimicrobials for infection prevention, treatment, and growth promotion (36,37). Consequently, it is essential to determine the susceptibility patterns of pathogenic microorganisms such as *P. aeruginosa* to guide judicious use of antibiotics and reduce the risk of promoting antibiotic resistance (38), and highlighting the importance of exploring other strategies to mitigate bacterial infections in poultry industry as feed additives and immunomodulatory substances (39-51).

In our study, we performed in vitro susceptibility testing to 12 antimicrobials and found a surprising resistance pattern among P. aeruginosa isolates. Remarkably, all isolates tested showed complete resistance to amoxicillin and cefotaxime. In addition, the results showed high levels of resistance to piperacillin96.66%, gentamicin86.66%, and ofloxacin80%. Different resistance levels were seen for cefepime and ceftazidime63.33% and levofloxacin and ciprofloxacin53.3%. In contrast, apramycin and doxycycline showed relatively low resistance rates36.66%, and amikacin had the highest sensitivity at 66.6%. Notably, most isolates96.66% resisted three or more antibiotics, indicative of multi-drug resistance. Furthermore, Isolates obtained from diseased birds showed significantly higher levels of resistance and lower susceptibility to most antibiotics than those obtained from apparently healthy birds. The exceptions were amoxicillin and cefotaxime, both isolates from diseased and healthy birds, which showed a resistance rate of 100%. Additionally, P. aeruginosa from apparently healthy birds are more resistant to apramycin, ofloxacin, and ceftazidime than P. aeruginosa isolated from diseased broiler chickens. This increased resistance poses significant risks, including treatment failure, economic losses, and public health concerns, as it may facilitate the transfer of resistance genes from animals to humans. Our findings on the resistance patterns of *P. aeruginosa* isolates are comparable with prior research. Badr et al. (32) found that while P. aeruginosa isolates were resistant to numerous antibiotics, they were responsive to levofloxacin. Elsayed et al. (2) found high levels of resistance to Amoxicillin and E-Moxclav among P. aeruginosa isolates and Jawher and Hassan (50) who reported 100% resistance to Amoxicillin among P. aeruginosa isolates. Elbehiriet al. (38) also assessed the antimicrobial resistance profiles of Pseudomonas isolates, finding resistance rates of 81.16% for nitrofurantoin, 71% for ampicillin and ampicillin/sulbactam, 65.22% for cefuroxime and ceftriaxone, and 55% for aztreonam, and found a resistance rate of 49.28% for ciprofloxacin. Furthermore, Oradyet al. (39) found a significant prevalence of resistance among P. aeruginosa isolates, with 90% resistant to ampicillin.

The findings suggest that *P. aeruginosa* demonstrates phenotypic multidrug resistance, probably controlled by genotypic factors such as antimicrobial resistance genes. Plasmids are particularly important in enabling the transfer of genes across different bacterial species. They can stimulate the emergence of new genetic variations and facilitate the exchange of major features, enhancing diversity in microbial communities (44). The transfer of antimicrobial genes across plasmids, known as Inter-plasmid antimicrobial gene transfer, is a significant mechanism that allows plasmids to acquire different antimicrobial resistance genes. This process contributes to understanding how multidrugresistant microbes develop and emerge (45). To further confirm the resistance profile of *P. aeruginosa* isolates in our study, we utilized PCR to examine the presence of five specific antimicrobial resistance genes (*mexR*, *arr*, *blaOXA*, *ampC*, and *ermB*) in 10 multidrug-resistant *P. aeruginosa* isolates. Our findings revealed that the *mexR* and *ampC* resistance genes were present in all isolates, while the *blaOXA*, *ermB*, and *arr* genes had incidence rates of 90%, 80%, and 50%, respectively. The results confirm the resistance profiles of the MDR *P. aeruginosa* obtained in this study, which aligns with previous research that has identified various antibiotic resistance genes in *P. aeruginosa* isolates as Hassan *et al.* (40) reported numerous antibiotic resistance genes, including *bla*_{CTX}, *fox*, and *mexR* in 100%, 80%, and 100% of the isolates, respectively.

Furthermore, Orady*et al.* (39) discovered resistance genes in *Pseudomonas* species isolates, including sul1, *bla_{TEM}*, *tetA*, *bla*_{CTX}-*M*, *blaOXA-1*, and *aadA1*, indicating the possibility of multidrug resistance. Similarly, Mohamed *et al.* (30) demonstrated antibiotic resistance in Pseudomonas isolates from chickens, notably within the β -lactamase family, and biofilm formation. Antibiotic resistance genes may explain phenotypic resistance to cephalosporin, β lactam, and other tested antimicrobials, raising the possibility of multidrug-resistant bacteria. In addition, these results highlight the effectiveness of PCR as a tool for detecting and validating antimicrobial resistance profiles in different microorganisms.

Although this study offered valuable insights about the antibiogram of P. aeruginosa in Fayoum Governorate in Egypt, it also displayed some limitations. Initially, the sample size was relatively small and restricted to broiler chickens from various farms within El-Fayoum Governorate, which may limit the applicability of the results to other regions or poultry populations. Furthermore, the study focused on a limited set of internal organs (liver, lung, gallbladder, kidney), which may not provide a comprehensive representation of the occurrence and dissemination of P. aeruginosa in other chicken tissues or systems (52).

Conclusion

In conclusion, our findings highlight a significant presence of *P. aeruginosa* in broiler chickens, accompanied by high levels of antimicrobial resistance and multiple resistance genes. These findings emphasize the urgent need for monitoring and controlling antimicrobial usage in poultry farms to mitigate the dissemination of multidrug-resistant. *aeruginosa* strains. Additionally, our findings suggest amikacin as a possibly effective treatment for *P. aeruginosa* infections.

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Conflict of interests

The author has no conflict of interest

References

- Dinev I, Denev S, Beev G. Clinical and morphological studies on spontaneous cases of *Pseudomonas aeruginosa* infections in birds. Pak Vet J. 2013;33:398–400. [available at]
- Elsayed MA, AmmarAM, Al Shehri ZS, Abd-El Rahman NA. Virulence repertoire of *P. aeruginosa* from some poultry farms with detection of resistance to various antimicrobials and plant extracts. Cell Mol Biol. 2016;62(1):124-132. DOI: <u>10.4172/1165-158X.1000124</u>
- Altaee AJ, Aldabbagh SY. Molecular identification of virulence genes of *Pseudomonas aeruginosa* isolated from fish (*Cyprinus carpio*) in Mosul city. Iraqi J Vet Sci. 2022;36(4):953-958. DOI: 10.33899/ijvs.2022.132660.2119
- Walker SE, Sander JE, Cline JL, Helton JS. Characterization of *P. aeruginosa* isolates associated with mortality in broiler chicks. Avian Dis. 2002;46(4):1045-50. DOI: <u>10.1637/0005-2086(2002)046(1045:COPAIA)2.0.CO;2</u>
- Jabbar LM, Abid TA. Treatment of infected wounds by using antimicrobial blue light phototherapy. Iraqi J Vet Sci. 2024;38(2),259-266. DOI: 10.33899/ijvs.2023.141837.3140
- Algammal AM, Eidaroos NH, Alfifi KJ, Alatawy M, Al-Harbi AI, Alanazi YF, Ghobashy MO, Khafagy AR, Esawy AM, El-Sadda SS, Hetta HF. opr L gene sequencing, resistance patterns, virulence genes, quorum sensing and antibiotic resistance genes of XDR *P. aeruginosa* isolated from broiler chickens. Infect Drug Resist. 2023;16:853–867. DOI: <u>10.2147/IDR.S401473</u>
- Al-Hiyali HM, Al-Kabbi HT, Abdulkarim S. Isolation of four types of bacteria that cause kidney damage in broiler chickens. Iraqi J Vet Med. 2005;29:33-42. DOI: <u>10.30539/iraqijvm.v29i1.860</u>
- John BH. Other bacterial disease: Pseudomonas. In: Calnek BW, John BH, Beard CW, Mcdougald LR, Saif YM, editors. Diseases of Poultry. 10thed. USA: John Wiley & Sons, Inc.; 1997. 291-292.
- Le Guern R, Grandjean T, Stabler S, Bauduin M, Gosset P, Kipnis É, Dessein R. Gut colonisation with multidrug-resistant *Klebsiella pneumoniae* worsens *P. aeruginosa* lung infection. Nat Commun. 2023;14(1):78. DOI: <u>10.1038/s41467-022-35767-4</u>
- Odoi H, Boamah VE, Boakye YD, Agyare C. Prevalence and phenotypic and genotypic resistance mechanisms of multidrug-resistant *P. aeruginosa* strains isolated from clinical, environmental, and poultry litter samples from the Ashanti region of Ghana. J Environ Public Health. 2021;9976064. DOI: <u>10.1155/2021/9976064</u>
- El-Oksh AS, Elmasry DM, Ibrahim GA. Effect of garlic oil nanoemulsion against multidrug resistant *Pseudomonas aeruginosa* isolated from broiler. Iraqi J Vet Sci. 2022;36(4),877-888. DOI: <u>10.33899/ijvs.2022.132430.2094</u>
- Jawher IM, Hasan MG. Molecular Identification of *Pseudomonas* aeruginosa in meat at Mosul city retails using PCR technique. Iraqi J Vet Sci. 2022;36(4):1083-1087. DOI: <u>10.33899/ijvs.2022.133086.2173</u>
- Langendonk RF, Neill DR, Fothergill JL. The building blocks of antimicrobial resistance in *P. aeruginosa* : Implications for current resistance-breaking therapies. Front Cell Infect Microbiol. 20211;1:665759. DOI: <u>10.3389/fcimb.2021.665759</u>
- Bush K, Bradford PA.β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med. 2016;6(8):a025247. DOI: <u>10.1101/cshperspect.a025247</u>
- 15. Marouf S, Li X, Salem HM, Ahmed ZS, Nader SM, Shaalan M, Awad FH, Zhou H, Cheang T. Molecular detection of multidrug-resistant *P. aeruginosa* of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against

multidrug-resistant *P. aeruginosa.* Poult Sci. 2023;102(10):102995. DOI: <u>10.1016/j.psj.2023.102995</u>

- Meng L, Liu H, Lan T, Dong L, Hu H, Zhao S, Zhang Y, Zheng N, Wang J. Antibiotic resistance patterns of *Pseudomonas spp*. isolated from raw milk revealed by whole genome sequencing. Front Microbiol. 2020;11:1005. DOI: <u>10.3389/fmicb.2020.01005</u>
- Nitz F, de Melo BO, da Silva LN, de Souza Monteiro A, Marques SG, Monteiro-Neto V, de Jesus Gomes Turri R, Junior AS, Conceição PR, Magalhães HC, Zagmignan A, Ferro TF, Bomfim MQ. Molecular detection of drug-resistance genes of *blaOXA-23-blaOXA-51* and mcr-1 in clinical isolates of *P. aeruginosa*. Microorganisms. 2021;9(4):786. DOI: <u>10.3390/microorganisms9040786</u>
- Vaillancourt M, Limsuwannarot SP, Bresee C, Poopalarajah R, Jorth P. *P. aeruginosa mexR* and mexEF antibiotic efflux pump variants exhibit increased virulence. Antibiotics. 2021;10(10):1164. DOI: 10.3390/antibiotics10101164
- Harris M, Fasolino T, Ivankovic D, Davis NJ, Brownlee N. Genetic Factors that contribute to antibiotic resistance through intrinsic and acquired bacterial genes in urinary tract infections. Microorganisms. 2023;11(6):1407. DOI: 10.3390/microorganisms11061407
- Wei Q, Ma LZ. Biofilm matrix and its regulation in *P. aeruginosa*. Int J Mol Sci. 2013;14(10):20983-1005. DOI: <u>10.3390/ijms141020983</u>
- Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, MacKie SA, editors. McCartney's practical medical microbiology.14thed. USA: Churchill Livingstone; 1996. 131–149 p.
- Quinn PJ, Markey BK, Carter ME, Donnelly WC, Leonard CF. Veterinary Microbiology and Microbial Disease. USA: Wiley-Blackwell; 2002.
- Khalafallah B, Abd El-Tawab A, Shaimaa S, Elkhayat M. Phenotypic and genotypic characterization of pseudomonas species isolated from frozen meat. Benha Vet Med J. 2020;39(2):47-51. DOI: 10.21608/bvmj.2020.46777.1285
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30thed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
- Jones C, Allsopp L, Horlick J, Kulasekara H, Filloux A. Subinhibitory concentration of kanamycin induces the *P. aeruginosa* type VI secretion system. PLoS One. 2013;8(11):e81132. DOI: 10.1371/journal.pone.0081132
- Sánchez P, Linares JF, Ruiz-Díez B, Campanario E, Navas A, Baquero F, Martínez JL. Fitness of in vitro selected *P. aeruginosa* nalB and nfxB multidrug resistant mutants. J Antimicrob Chemother. 2002;50(5):657-64. DOI: <u>10.1093/jac/dkf185</u>
- Schwartz T, Kohnen W, Jansen B, Obst U. Detection of antibioticresistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol Ecol. 2003;43(3):325–335. DOI: <u>10.1111/j.1574-6941.2003.tb01073.x</u>
- Colom K, Pèrez J, Alonso R, Fernández-Aranguiz A, Lariňo E, Cisterna R. Simple and reliable multiplex PCR assay for detection of *bla*TEM, blaSHV and *blaOXA*-1 genes in Enterobacteriaceae. FEMS Microbiol Lett. 2003;223(2):147-151. DOI: <u>10.1016/S0378-1097(03)00306-9</u>
- Nguyen MC, Woerther PL, Bouvet M, Andremont A, Leclercq R, Canu A. *Escherichia coli* as reservoir for macrolide resistance genes. Emerg Infect Dis. 2009;15(10):1648-50. DOI: <u>10.3201/eid1510.090696</u>
- Mohamed HA, Alnasser SM, Abd-Elhafeez HH, Alotaibi M, Batiha GE, Younis W. Detection of β-lactamase resistance and biofilm genes in Pseudomonas species isolated from chickens. Microorganisms. 2022;10(10):1975. DOI: <u>10.3390/microorganisms10101975</u>
- Hai-ping HE. Isolation and identify of *P. aeruginosa* in chicken deadembryos. Chinese Qinghai J Anim Vet Sci. 2009;3:25-27.
- Badr H, Roshdy H, Abd El-Hafez SA, Farghaly EM. Prevalence, pathogenicity, and antibiogram sensitivity of *P. aeruginosa* isolated from diseased chickens. Assiut Vet Med J. 2016;62(151):119-126. DOI: <u>10.21608/AVMJ.2016.170015</u>
- Abd El-Hafeez RI, Ahmed AB, Hassan AS. Phenotypic and genotypic characterization of *P. aeruginosa* recovered from kidney lesions of broiler chickens. Assiut Vet Med J. 2018;64(156):110-6. DOI: 10.21608/AVMJ.2018.168706

- 34. Ohore OG, Jubril AJ, Adekunle LA. Haematology and pathologic changes associated with *P. aeruginosa* isolated from barn swallows around poultry houses in broiler chickens. Sokoto J Vet Sci. 2022;20(1):26-34. DOI: <u>10.4314/sokjvs.v20i1.4</u>
- Bennani H, Mateus A, Mays N, Eastmure E, Stärk KD, Häsler B. Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. Antibiotics. 2020;9(2):49. DOI: 10.3390/antibiotics9020049
- Laxminarayan R, Van Boeckel T, Teillant A. The economic costs of withdrawing antimicrobial growth promoters from the livestock sector. OECD Food Agric Fish Papers. 2015;78. DOI: <u>10.1787/5js64kst5wvlen</u>
- Abdulrazaq H, Ameen Q. Genetic relationship between local guinea fowl, quail and chicken using RAPD–PCR technique. Mesopotamia J Agric. 2023;51(4):39-49. DOI: <u>10.33899/mja.2023.142638.1265</u>
- Elbehiry A, Marzouk E, Aldubaib M, Moussa I, Abalkhail A, Ibrahem M, Hamada M, Sindi W, Alzaben F, Almuzaini AM, Algammal AM, Rawway M. Pseudomonas species prevalence, protein analysis, and antibiotic resistance: An evolving public health challenge. AMB Express. 2022;12(1):53. DOI: <u>10.1186/s13568-022-01390-1</u>
- Orady RM, Matter AA, Ebrahem AF. In-vitro inhibition of biofilm formation by *P. aeruginosa* isolated from chicken. Alex J Vet Sci. 2022;74(1). DOI: <u>10.5455/ajvs.44222</u>
- Hassan WH, Ibrahim AK, Shany SS, Salam HH. Virulence and resistance determinants in *P. aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt. J Adv Vet Anim Res. 2020;7(3):452-463. DOI: <u>10.5455/javar.2020.g441</u>
- 41. Widodo A, Lamid M, Effendi MH, Khairullah AR, Riwu KH, Yustinasari LR, Kurniawan SC, Ansori AN, Silaen OS, Dameanti FN. Antibiotic sensitivity profile of multidrug-resistant (MDR) Escherichia coli isolated from dairy cow's milk in Probolinggo, Indonesia. Biodiversitas. 2022;23(10):4971-4976. DOI: <u>10.13057/biodiv/d231002</u>
- Khayoon T, Abbas R, Abdullah F. Effects of feeding various levels of postbiotics produced by lactic acid bacteria on growth performance, gastrointestinal microbiota count, and digestibility of some nutrients in broiler chickens. Mesopotamia J Agric. 2024;52(2):68-81. DOI: 10.33899/mja.2024.145531.0101329
- Sabdoningrum EK, Hidanah S, Ansori AM, Fadholly A. Immunomodulatory and antioxidant activities of *Phyllanthus niruri* L. extract against the laying hens infected by *Escherichia coli*. Res J Pharm Tech. 2020;13(5):2246-2250. DOI: <u>10.5958/0974-360X.2020.00404.7</u>
- Bottery MJ. Ecological dynamics of plasmid transfer and persistence in microbial communities. Curr Opin Microbiol. 2022;68:102152. DOI: 10.1016/j.mib.2022.102152
- Wang X, Zhang H, Yu S, Li D, Gillings MR, Ren H, Mao D, Guo J, Luo Y. Inter-plasmid transfer of antibiotic resistance genes accelerates antibiotic resistance in bacterial pathogens. ISME J. 2024;18(1):032. DOI: <u>10.1093/ismejo/wrad032</u>
- Alkhashb A, Alhaji T, Thalij K. Effectiveness of chitosan and agnanoparticle films on the quality of chicken meat. Mesopotamia J Agric. 2024;52(2):14-26. DOI: <u>10.33899/mja.2024.145729.011337</u>
- Hidanah S, Sabdoningrum EK, Arif MA, Ansori AM, Hasanah TP, Widaya LA. Sambiloto (*Andrographis paniculata*) extract improves the performance of animal model infected with *Escherichia coli*. Indian J Forensic Med Toxicol. 2020;14(4):3491-3496. DOI: 10.37506/ijfmt.v14i4.12167
- Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F. *P. aeruginosa* : Resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect. 2007;13(6):560-78. DOI: <u>10.1111/j.1469-0691.2007.01681.x</u>
- Mahendra MY, Purba RA, Dadi TB, Pertiwi H. Estragole: A review of its pharmacology, effect on animal health and performance, toxicology, and market regulatory issues. Iraqi J Vet Sci. 2023;37(2),537-546. DOI: <u>10.33899/ijvs.2022.135092.2445</u>
- Jawher IM, Hasan MG. Antibiotics resistance patterns of *Pseudomonas* aeruginosa isolated from meat at Mosul city retails. Iraqi J Vet Sci. 2023;37(2):363-367. DOI: <u>10.33899/ijvs.2022.133961.2322</u>

العزل والتوصيف الجزيئي للزائفة الزنجارية المقاومة للأدوية المتعددة المعزولة من الدجاج اللاحم في الفيوم بمصر

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الخلاصة

في صناعة الدواجن، تكتسب بكتريا الزائفة الزنجارية سالبة الجرام أهمية كممرض انتهازي ناشئ له آثار سريرية ملحوظة. هذه البكترياً الممرضة سالبة الجرام لديها القدرة على تلويث المفرخات، مما يؤدى إلى مجموعة من الأعراض التنفسية الشديدة، والتهاب الأمعاء، وتسمم الدم، والتهاب القرنية، والتهاب الجيوب الأنفية، والتهاب السمف، والكلية، والإصابات والوفيات السريعة، مما يشير إلى الإمكانات المسببة للأمراض المتنوعة لهذه البكتريا الممرضة الطيور. من خلال هذه الدراسة، تم جمع ما مجموعه ٤٨٠ عينة (١٢٠ لكل منها) من الكبد والرئة والمرارة والكلي من دجاج التسمين من مختلف الأعمار والفحص البكتيري. تراوحت معدلات العزل الإجمالية ل للزائفة الزنجارية من ٨, ٧٠٪ إلى ٨٣,٣٪. ظاهريا، كشف اختبار مقاومة المضادات الحيوية للعز لات المختارة (العدد = ٣٠) أن (٩٦,٦٦٪ كانت مقاومة لثلاثة مضادات حيوية أو أكثر من مجموعات مختلفة من مضادات الميكر وبات، مما يشير إلى مقاومة الأدوية المتعددة، والتي كانت أعلى مقاومة لها للأموكسيسيلين ١٠٠٪، والبيبير اسيلين ٩٦,٦٦٪، والجنتاميسين ٨٦,٦٦٪ أوفلوكساسين ٨٠٪، والسيفيبيم ٦٣,٣٣٪، والسيفتازيديم ٦٣,٣٣٪، والليفو فلوكساسين ٥٣,٣٪ وسيبر وفلوكساسين ٥٣,٣٪ يليه أبر امايسينو دوكسيسيكلين ٣٦,٦٦٪ لكل منهما بينما كانت ٣٦,٦٦٪ من العز لات حساسة للأميكاسين. ور اثيا، تم استخدام تفاعل البلمرة المتسلسل لتحديد خمس جينات مقاومة في عشرة عزلات مختارة من للزائفة الزنجارية متعددة المقاومة للمضادات الحيوية، وكشفت النتيجة أن ٠٠٠٪ من العزلات المختبرة تحتوي على جينات مقاومة mexR و ermB و blaOXA و blaOXA و ampC و۹.arr و ۸۰ و ۰۰٪ على التوالي.

- Hanoush N. Honey as antibacterial agent used against *Bacillus spp.* isolated from locally produced juice. Mesopotamia J Agric. 2023;51(4):72-85. DOI: <u>10.33899/mja.2023.143201.1273</u>
- Jawher IM, Hasan MG. Detection of some virulence genes of *Pseudomonas aeruginosa* isolated from meat at Mosul city. Iraqi J Vet Sci. 2022;36(I):101-105. DOI: <u>10.33899/ijvs.2022.135755.2512</u>