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Incidence and virulence gene profiling of *Pseudomonas aeruginosa* in broiler chickens from Fayoum Governorate, Egypt

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ABSTRACT

Pseudomonas aeruginosa is a serious bacterial disease that causes significant losses in the poultry industry. This pathogen also exhibits zoonotic importance and is associated with severe human infections such as pulmonary cystic fibrosis and urinary tract infections. The pathogenicity of *Pseudomonas aeruginosa* bacteria is primarily due to the production of extracellular enzymes and the expression of virulence factors, such as *tox*A and *opr*L. The molecular identification of these genes can help in the early detection and control of infections caused by this bacterium. Here, 480 samples were collected from different organs (lung, kidney, gall bladder, and liver) of broiler chickens from four cities in Fayoum governorate including, Ibshaway, Tamyyah, Itsa and Sinnuris. The bacteriological analysis revealed that 369 out of the 480 samples were positive for *P. aeruginosa* with an incidence rate of 76.87%, of which the highest isolation rate was from Tamyyah (90.83%), followed by Ibshaway (85.0%), Itsa (75.92%), and Sinnuris (52.67%). Furthermore, virulence gene profiling of eight selected isolates using polymerase chain reaction (PCR) indicated that all eight tested isolates (100%) were positive for the harbored *opr*L gene, whereas only five *P. aeruginosa* isolates (62.5%) were positive for *the tox*A gene. In conclusion, the widespread occurrence of this opportunistic pathogen in poultry production systems is particularly concerning, as it suggests that *P. aeruginosa* is becoming a major concern for poultry health and food safety in the studied cities.

1. INTRODUCTION

Pseudomonas is a genus of Gram-negative, aerobic, rod-shaped bacteria that are commonly found in various environments. They are recognized for their versatile metabolic abilities, their capacity to inhabit a range of ecological settings, and their potential to cause infections in both humans and animals (Freschi et al., 2019). Among the species in this genus, *Pseudomonas aeruginosa* is particularly notable and clinically significant.

Pseudomonas aeruginosa is a serious disease-causing organism that affects most types of birds at any age and causes huge economic losses in the poultry sector. It is considered a bacterial hazard that contaminates hatcheries and causes severe respiratory symptoms, enteritis, septicaemia, keratitis, sinusitis and omphalitis, as well as high death rate at any age.

P. aeruginosa is an opportunistic pathogen capable of infecting a variety of hosts, including animals, and humans. It is notable for its inherent resistance to many antibiotics, which poses a considerable challenge in medical environments and is marked by its unique green-blue coloration (Qin et al., 2022). *P. aeruginosa* of avian origin is often characterized by its high resistance to clinically important antibiotics due to the extensive use of antimicrobial agents in poultry production. Its spread in poultry threatens public health, especially those with compromised immunity, as it is one of the major causes of lung infections in people with cystic fibrosis. This bacterium produces numerous virulence factors, such as exotoxins, proteases, and hemolysins, which play a role in its ability to cause disease (Liao et al., 2022).

Pseudomonas infections in poultry, particularly *P. aeruginosa*, are of great concern to the poultry industry. *P. aeruginosa* can cause a wide range of diseases in chickens, leading to significant economic losses. Moreover, *P. aeruginosa* is considered a zoonotic pathogen, posing a potential public health risk. People with weakened immune systems, including the elderly, young children, or individuals with pre-existing medical conditions, are especially susceptible to *Pseudomonas* infections, which can result in severe complications (Hassanain et al., 2013).

The pathogenicity of *P. aeruginosa* is attributed to its production of various extracellular virulence factors, including exotoxin A, elastase, and proteases. These factors help the bacterium evade the host's immune system, damage host tissues, and establish infections. Notably, the *tox*A and *opr*L genes are of particular interest. Exotoxin A (*tox*A) is a powerful cytotoxin that inhibits protein synthesis in host cells and is a major factor in *P. aeruginosa*'s pathogenicity (Jurado-Martín et al., 2021). Additionally, the outer membrane protein *opr*L contributes to the bacterium's antibiotic resistance and immune evasion. The *opr*L gene encodes this lipoprotein, which is crucial for maintaining the structural integrity of the bacterial cell wall and plays a role in antibiotic resistance (Rodríguez-Herva et al., 1996).

Polymerase chain reaction (PCR) is a precise and highly sensitive technique used to identify the presence of specific

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virulence genes in different bacterial species (El-Tawab et al., 2021). The use of PCR for the identification of some virulence genes such as *tox*A and *opr*L can help in the early detection and control of infections caused by *P. aeruginosa* and can ultimately help to reduce economic losses and improve food safety in the poultry industry (Salman et al., 2013).

The aim of this study is to emphasize the potential risk of *P. aeruginosa* outbreaks in poultry flocks within El Fayoum governorate, Egypt, as well as the detection of some virulence genes such as *tox*A and *opr*L genes that contribute to the high isolation rates using PCR

2. MATERIAL AND METHODS

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

2.1. Sampling

A total of 480 samples were collected from different internal organs (lung, kidney, gall bladder, and liver) of broiler chickens representing four cities of El-Fayoum Governorate divided as the following 140 sample collected from Ibshaway, 120 from Tamyyah, 112 from Sinnuris and 108 from Itsa. The samples were collected aseptically from diseased, freshly dead, and apparently healthy chickens. Then, samples were transported in ice boxes and submitted for bacteriological examination and isolation of *P. aeruginosa*. The prominent feature of the disease in affected farm has been associated with high mortality, particularly in newly hatched chickens. Depression, loss of appetite, rough coat, standing with closed eyes, and death were also observed in most infected chickens by the disease.

2.2. Isolation and Biochemical Identification of P. aeruginosa

The gathered samples were cultured separately in nutrient broth (Oxoid) and incubated for 24 h at 37°C for primary enrichment. A loopful of broth was spread on Cetrimide agar (Oxoid) followed by incubation under aerobic conditions at 37°C for 24h. The isolates were presumptively identified as *P. aeruginosa* based on cultural characteristics and biochemical tests. Furthermore, *P. aeruginosa* could be identified by its characteristic production of the blue-green pigment pyocyanin and its characteristic grape-like odor, and biochemical tests including oxidase, catalase, Indol, Methyl Red (MR) and Voges-Proskauer (VP) and urease (Collee et al., 1996; Quinn et al., 2002; Khalafallah et al., 2020).

2.3. Bacterial Preservation

Single colonies showing characteristic colonial appearance and morphological features were selected and transferred to 0.5% semisolid agar. These were then incubated at 37°C for 24h and subsequently stored at 4°C. In addition, a 20% glycerol culture broth was stored at −20°C (Collee, et al. 1996).

2.4. Molecular identification of P. aeruginosa virulence genes

The molecular identification of virulence genes in *P. aeruginosa* was conducted using polymerase chain reaction (PCR) targeting two virulence genes: *opr*L and *tox*A.

Eight representative *P. aeruginosa* strains (n=8) were selected for genotypic virulence screening.

Genomic DNA was extracted from confirmed cultures using the QIAamp DNA Extraction Miniprep Kit, following the manufacturer's guidelines. Oligonucleotide primer sequences and amplified product sizes are shown in Table (1). PCR conditions were performed according to the protocols described in the specified references.

Table 1 Oligonucleotide primers sequences

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
*opr*L	F: ATG GAA ATG CTG AAA TTC GGC R: CTT CTT CAG CTC GAC GCG ACG	504 bp	Xu et al., 2004
*tox*A	F: GAC AAC GCC CTC AGC ATC ACC AGC R: CGC TGG CCC ATT CGC TCC AGC GCT	396 bp	Matar et al., 2002

3. RESULTS

3.1 Bacteriological and biochemical identification of P. aeruginosa

The *P. aeruginosa* colonies typically appeared as large, smooth, and glistening, with the characteristic blue-green pigment of *P. aeruginosa* diffusing into the surrounding medium. In addition, *P. aeruginosa* exhibited positive results for Oxidase, Catalase and Urease, while negative results for Indol, Methyl Red (MR) and Voges-Proskauer (VP) tests.

3.2 Incidence of *P. aeruginosa* among broiler chicken collcetd from different cities in El-Fayoum Governorate

Out of 480 samples, 369 tested positive for *P. aeruginosa*, representing an incidence of 76.87%. Among the four cities tested, the highest isolation rate was found in Tamyyah at 90.83%, followed by Ibshaway at 85.0%, then Itsa at 75.92%, and Sinnuris at 52.67% (Table 2).

3.2 Prevalence of oprL amd toxA genes among *P. aeruginosa* isolates

A virulence gene profiling of eight selected isolates was done using polymerase chain reaction (PCR) targeting the oprL and toxA genes with results indicating that all the tested eight isolates (n=8) harbored *opr*L gene in a percentage of 100%, whereas only five out of the eight *P. aeruginosa* isolates under investigation were positive for *tox*A gene in a percentage of 62.5% (Table 3, Figure 1).

Table 2 Total number of the collected samples from different cities of El-Fayoum Governorate and the incidence rates of *P. aeruginosa* recovered from the examined samples

City	No. of collected samples	No. of Positive Samples	Percentage of positive samples (%)
Ibshaway	140	119	85.0%
Tamyyah	120	109	90.83%
Itsa	108	82	75.92%
Sinnuris	112	59	52.67%
Total	480	369	76.87%

Table 3 Prevalence of *opr*L and *tox*A virulence genes among the examined *P. aeruginosa* isolates

Sample	*tox*A	*opr*L
1	+	+
2	-	+
3	+	+
4	+	+
5	-	+
6	+	+
7	-	+
8	+	+
Percentage (%)	5/8 (62.5%)	8/8 (100%)

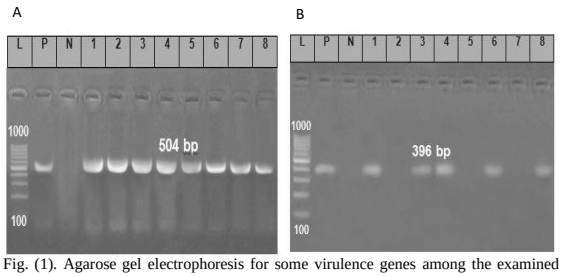

Fig. (1). Agarose gel electrophoresis for some virulence genes among the examined *P. aeruginosa* isolates. Lanes 1-10: DNA marker, L; 100-1000 bp DNA ladder, P: Positive control (*Pseudomonas aeruginosa* (ATCC® 27853™)), N: Negative control. (A) Lane 1-8 positive at 504 bp for *opr*L gene for the examined *P. aeruginosa* isolates. (B) Lane 1,3,4,6,8 positive at 396bp for *tox*A gene of the examined *P. aeruginosa* isolates

4. DISCUSSION

P. aeruginosa infection can occur in broiler chickens of any age, but it can be particularly harmful to young birds. Infection can occur through mechanical means, such as skin injuries or the use of contaminated needles during vaccination. Factors like the bird's immune status and the presence of other simultaneous infections can heighten susceptibility to *P. aeruginosa* infection (Labib and Roshdy, 2021).

In the chicken industry, *P. aeruginosa* is a serious bacterium that can cause septicaemia, lung infections, and yolk sac infections, among other diseases. This bacterium has the potential to cause significant financial losses due to their capacity to lower growth rates, raise mortality, and lower egg production (Walker, et al., 2002). Moreover, *P. aeruginosa* is a zoonotic infection, which implies that humans can get it from animals. This is especially worrying for the poultry sector since chickens may function as a reservoir for the bacteria and spread it to humans by eating contaminated poultry products or coming into close contact with affected birds (Abd El-Ghany, 2021).

In order to report the incidence rate of *P. aeruginosa*, 480 samples were collected from various internal organs of broiler chickens from four different cities in El-Fayoum Governorate, Egypt then bacteriologically examined for the incidence of *P. aeruginosa*. The results of this study revealed a high incidence of *P. aeruginosa* (76.87 %) among broiler chickens, which may be attributed to the ubiquitous nature of this opportunistic pathogen in the poultry farm environment and the potential for cross-contamination between different production stages and thus highlighted the need for improved biosecurity measures and hygiene practices, effective control strategies, and prudent use of antibiotics to mitigate the impact of this highly prevalent pathogen on poultry production and food safety.

The previously stated research demonstrated the remarkably different frequencies of *P. aeruginosa* in broiler chicken in Egypt , such as (Abd-El Gwad et al., 1998) who found that only 16 out of 200 freshly dead growing chickens collected from different governmental farms at Assiut Governorate were positive cases of *P. aeruginosa* with an incidence of 8%. Badr et al. (2016) reported the prevalence of *P. aeruginosa* as 6.5% in the examined samples, out of them 7.3% in the diseased chicken and 4% in one-day-old chicks. Tawakol et al. (2018) also isolated and identified *P. aeruginosa* from 150 diseased broiler chickens in Dakahlia Governorate and found that 16 isolates were positive with an incidence of (10.66%). Hassan et al. (2020) reported that 45 out of 250 collected samples from the cases of pericarditis in broiler chickens were positive for *P. aeruginosa* with prevalence of 18%. The latest study by Algammal et al. (2023), which analyzed 200 samples from 120 broiler chickens in farms located in Ismailia Governorate, Egypt, found that the overall prevalence of *P. aeruginosa* among the examined birds was 28.3%. In addition, , the results revealed a diifrence in the incidence of *P. aeruginosa* among the four cities under investigation with the highest incidence rate reported in Tamyyah city (90.83%) followed by Ibshaway, Itsa and Sinnuris with incidence rates of 85.0%, 75.92% and 52.67%, respectively. The difference in incidence rates, between our sudy and the different studies, in addition to the difference in incedince rates among cities in our study might be attributed to difference in biosecurity measures, farm management practices, and the implementation of effective control strategies in the studied cities. Additionally, differences in sampling methods, diagnostic techniques, and the specific populations of poultry examined could also contribute to the variation in isolation rates.

Phenotypic approaches have been the conventional way of identifying *P. aeruginosa*. The *opr*L gene is a widely used molecular marker for the detection and identification of *P. aeruginosa* as it encodes an outer membrane lipoprotein that is specific to this species (Nikbin et al., 2012).

In the present study, determining the virulence gene profiles of eight selected isolates using polymerase chain reaction (PCR) indicated that all the retrieved selected isolates were tested positive for the *opr*L gene.

The higher occurrence of the *opr*L gene in this study suggests a high degree of genetic conservation and species-specificity of this gene among the *P. aeruginosa* isolates in the studied poultry farms, and this finding could be useful in the development of reliable molecular detection and identification methods for *P. aeruginosa* in the poultry industry, particularly in regions where this pathogen is highly prevalent.

The *tox*A gene in *P. aeruginosa* encodes the exotoxin A, which is a major virulence factor produced by this opportunistic pathogen. Exotoxin A is known to inhibit host protein synthesis, leading to cell death and contributing to the pathogenicity of *P. aeruginosa* infections (Qin et al., 2022).

In the present study, five out of eight selected isolates of the *P. aeruginosa* tested positive for the *tox*A gene. The high prevalence of the *tox*A gene among *P. aeruginosa* isolates from poultry is particularly concerning, as exotoxin A is a potent virulence factor that can contribute to severe disease manifestations in infected birds.

Several recent studies have reported a relatively high prevalence of the *opr*L and *tox*A genes among *P. aeruginosa* isolates obtained from poultry samples. In 2019, the molecular features of *P. aeruginosa* collected from several hatcheries and farms throughout the Luxor governorate revealed that all the examined isolates possessed the *opr*L gene (100%), while the incidence of the *tox*A gene was 71.42% (Shahat et al., 2019).

Also, Bakheet and Torra (2020) performed PCR testing on 24 isolates of *P. aeruginosa* that were derived from poultry origin and discovered that the *opr*L gene had a high distribution (100%) compared to the distribution of other virulence genes, such as *tox*A, which were 83.3%. El-sadda et al. (2021) conducted a PCR assay to identify virulence genes such as *opr*L and *tox*A in *P. aeruginosa* isolates from broiler chickens of various ages. They found that the *opr*L gene was present in 100% of the samples, while the *tox*A gene was detected in 80% of the samples.In a recent study by Algammal et al. (2023) on *P. aeruginosa* isolated from broiler chickens on farms in Ismailia Governorate, Egypt, the overall prevalence of the virulence-related genes *opr*L and *tox*A was found to be 100% for both genes.

5. CONCLUSIONS

This study demonstrates a high incidence of *Pseudomonas aeruginosa* among broiler chickens in Fayoum Governorate, Egypt with the highest isolation rate observed in birds from Tamyyah city. In addition, the study revealed high prevalence rates of the *opr*L and *tox*A virulence genes among the examined isolates, indicating significant public health and economic risks. These findings highlight the urgent need for robust control strategies to mitigate the widespread incidence of the *P. aeruginosa* species in poultry industry and protect both animal welfare and public health from risks posed by these virulent, toxin-producing strains.

6. REFERENCES

1. Abd El-Ghany, W.A., 2021. *Pseudomonas aeruginosa* infection of avian origin: Zoonosis and one health implications. Vet World., 14,8, 2155-2159.
2. Abd-El Gwad, A.M., Aly, S.M., Azzaz, H.A., 1998. Some studies on pseudomonas infections in growing chickens in Assiut Farms. Assiut Veterinary Medical Journal, 38,76, 90-97.
3. Algammal, A.M., Eidaroos, N.H., Alfifi, K.J., Alatawy, M., Al-Harbi, A.I., Alanazi, Y.F., El-Tarabili, R.M., 2023. Opr l gene sequencing, resistance patterns, virulence genes, quorum sensing and antibiotic resistance genes of xdr *Pseudomonas aeruginosa* isolated from broiler chickens. Infect Drug Resist., 13, 16:853-867.
4. Badr, H., Roshdy, H., Abd El-Hafez, S.A.E.H., Farghaly, E.M., 2016. Prevalence, pathogenicity and antibiogram sensitivity of *Pseudomonas aeruginosa* isolated from diseased chickens. Assiut Veterinary Medical Journal, 62,151, 119-126.
5. Bakheet, A.A. and Torra, D.E., 2020. Detection of *Pseudomonas aeruginosa* in dead chicken embryo with reference to pathological changes and virulence genes.Alexandria Journal of Veterinary Sciences, 65,1, 81-89.
6. Collee, J.G., Miles, R.S., Watt, B., 1996. Tests for identification of bacteria. In MacKie and McCartney's practical medical microbiology,14th ed. Edited by Collee, J. G.; Fraser A. G.; Marmion,B. P. and Simmons, A. New York: Churchill Livingstone. pp. 131–149.
7. El-sadda, S.S., Esawy, A., ELTarabili, R.M., Khafagy, A., 2021. Molecular characterization and genetic analysis of *Pseudomonas aeruginosa* recovered from broiler chickens. Suez Canal Veterinary Medical Journal. SCVMJ, 26,1, 61-77.
8. El-Tawab, A.A.A., El-Hofy, F.I., Hasb-Elnaby, G.R., El-Khayat, M.E., Refaey, M.A., 2021. Prevalence and virulence genes of vibrio and Aeromonas species isolated from Nile tilapia and mugil fish farms in Egypt. Adv. Anim. Vet. Sci., 9,10, 1625-1631.
9. Freschi, L., Vincent, A.T., Jeukens, J., Emond-Rheault, J.G., Kukavica-Ibrulj, I., Dupont, M.J., Levesque, R.C., 2019. The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer, and pathogenicity. Genome Biology and Evolution, 11,1, 109-120.
10. Hassan, W.H., Ibrahim, A.M.K., Shany, S.A.S., Salam, H.S.H., 2020. Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt. Journal of advanced veterinary and animal research, 7,3, 452–463.
11. Hassanain, N.A., Hassanain, M.A., Ahmed, W.M., Shaapan, R.M., Barakat, A.M., El-Fadaly, H.A., 2013. Public health importance of foodborne pathogens. World Journal of Medical Sciences 9,4, 208-222.
12. Jurado-Martín, I., Sainz-Mejías, M., McClean, S., 2021. *Pseudomonas aeruginosa*: an audacious pathogen with an adaptable arsenal of virulence factors. International journal of molecular sciences 22,6, 3128.
13. Khalafallah, B., Abd El-Tawab, A., Shaimaa, S., Elkhayat, M. 2020. Phenotypic and genotypic characterization of pseudomonas species isolated from frozen meat. Benha Veterinary Medical Journal 39,2, 47-51
14. Labib, M. and Roshdy, H., 2021. Potential risk of pseudomonas infection in broiler chickens with detection of the antibiotic resistant genes. Egyptian Poultry Science Journal, 40,4, 783-792.
15. Liao, C., Huang, X., Wang, Q., Yao, D., Lu, W., 2022. Virulence factors of *Pseudomonas aeruginosa* and antivirulence strategies to combat its drug resistance. Frontiers in cellular and infection microbiology 12, 926758.
16. Matar, G.M., Ramlawi, F., Hijazi, N., Khneisser, I., Abdelnoor, A.M., 2002. Transcription Levels of *Pseudomonas aeruginosa*Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. Curr Microbiol., 45,5, 350-354.
17. Nikbin, V.S., Aslani, M.M., Sharafi, Z., Hashemipour, M., Shahcheraghi, F., Ebrahimipour, G.H., 2012. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. Iran J Microbiol., 4,3, 118-123.
18. Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., Wu, M., 2022. *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal transduction and targeted therapy, 7,1, 199.
19. Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., 2002. Veterinary Microbiology and Microbial Disease. Iowa State University Press, Ames, Iowa, USA, 2002. 536 pp. ISBN 0-632-05525-1.
20. Rodríguez-Herva, J.J., Ramos-Gonzalez, M.I., Ramos, J.L., 1996. The Pseudomonas putida peptidoglycan-associated outer membrane lipoprotein is involved in maintenance of the integrity of the cell cell envelope. Journal of Bacteriology *178*,6, 1699-1706.
21. Salman, M., Ali, A., Haque, A., 2013. A novel multiplex PCR for detection of *Pseudomonas aeruginosa*: A major cause of wound infections. Pak J Med Sci., 29,4, 957-961.
22. Shahat, H.S., Mohamed, H., Al-Azeem, A., Mohammed, W., Nasef, S.A., 2019. Molecular detection of some virulence genes in *Pseudomonas aeruginosa* isolated from chicken embryos and broilers with regard to disinfectant resistance. SVU-International Journal of Veterinary Sciences, 2,2, 52-70.
23. Tawakol, M., Nabil, N., Reda, R., 2018. Molecular studies on some virulence factors of *Pseudomonas aeruginosa* isolated from chickens as a biofilm forming bacteria. Assiut Veterinary Medical Journal, 64,159, 43-51.
24. Walker, S.E., Sander, J.E., Cline, J.L., Helton, J.S., 2002. Characterization of *Pseudomonas aeruginosa* isolates associated with mortality in broiler chicks. Avian Diseases, 46,4, 1045-1050.
25. Xu, J., Moore, J.E., Murphy, P.G., Millar, B.C., Elborn, J.S. 2004. Early detection of *Pseudomonas aeruginosa* – comparison of conventional versus molecular ,(PCR) detection directly from adult patients with cystic fibrosis ,CF). Ann Clin Microbiol Antimicrob., 20, 3:21.