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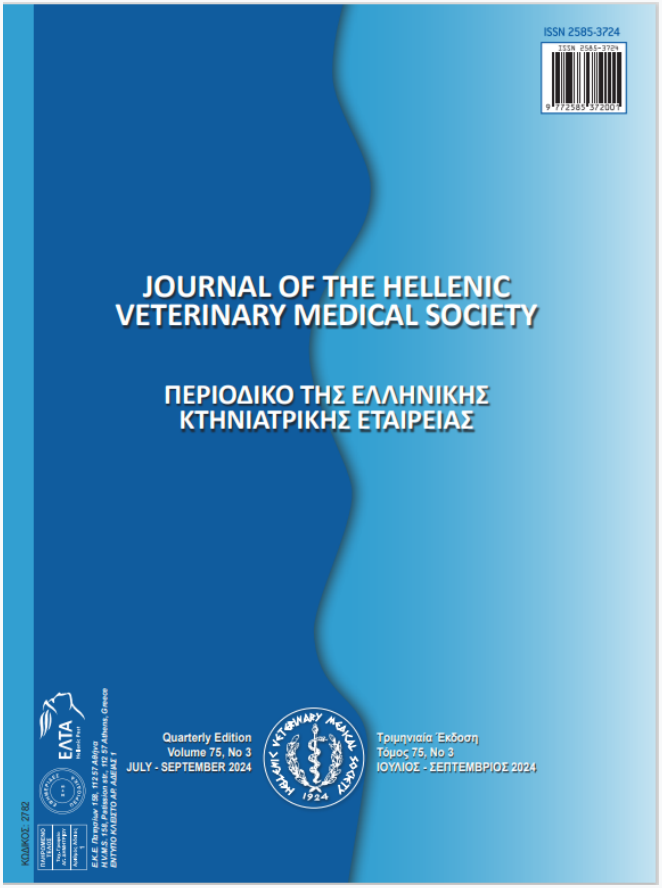

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Research article
Ερευνητικό άρθρο

Genetic profiling of virulence genes among *Pseudomonas Aeruginosa* isolates recovered from diseased chickens

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ABSTRACT: *Pseudomonas aeruginosa* (P. *aeruginosa*) is one of the opportunistic organisms. It holds multiple factors incorporated into virulence and pathogenicity producing severe economic losses in poultry farms. This study aimed to investigate the prevalence of *Pseudomonas aeruginosa* retrieved from different chicken farms and find out their serogroups, antimicrobial-resistant pattern and the occurrence of the virulent genes using polymerase chain reaction (PCR) against six virulent genes (*lasL, lasB toxA, exoU, exoT,* and *oprl* genes). Two hundred cases suffered from some respiratory manifestation and septicemic picture in addition to yolk sacs from one-day-old chicks. All samples were examined bacteriologically, serologically and molecularly. The isolated strains were tested against eight antimicrobial discs. Eighteen isolates out of 200 (9%) of *P. aeruginosa* were defined. Serologically the isolates were related to more serogroup (O1, O4, O6, O11, and O12) P. *aeruginosa*. All isolates showed multidrug resistance (MDR) to more than one of antimicrobial discs such as (sulfamethoxazole, erythromycin, tetracycline, and ampicillin). However, those isolates exhibited different sensitivities as 100% and 22.2% to ciprofloxacin and norfloxacin respectively. By PCR, all *P. aeruginosa* strains carried four virulence genes (*lasI, toxA, exoU,* and *oprl*). However, there were different ratios of detection for *lasB,* and *exoT* genes. In conclusion, presence of different factors of virulent genes (*lasI, toxA, exoU,* and *oprl*) which were predominant in all strains of *P. aeruginosa*. The MDR to more than one antimicrobial drug. Make some restrictions in the treatment. *Pseudomonas* infection in poultry requires strict biosecurity measures to control and eradicate the infection by *P. aeruginosa;*

Keywords: *Pseudomonas; aeruginosa;* 16SRNA; virulence genes; Quorum sensing (QS); poultry

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INTRODUCTION

Pseudomonas is one of the persistent agents in many living organisms, food, and the environment (Ven-Heir et al., 2021). Normally, *P. aeruginosa* is considered normal flora in bird species. Inversely, under stress factors, the organism becomes pathogenic. The infection is associated with some symptoms, like respiratory signs (nasal discharge-swollen sinuses- gasping) and developing septicemia, and diarrhea, (Gong et al*.,* 2018). In the poultry industry, *Pseudomonas spp*. has been the main organism concerned with chicken bodies (Chen et al., 2020). The zoonotic nature of the organism was reported, especially in one-day-old poultry, with significant losses (Abd El Ghanyet et al., 2021). Products from chicken are implicated as a major source of *Pseudomonas spp.* The human disease may progress into a severe lung disorder (Azam and Khan 2019). *P. aeruginosa* produces β-hemolysis on blood agar plates and grows in aerobic conditions. It is difficult to treat *P. aeruginosa* due to some inherent factors that have developed antibiotic resistance (Lister *et al.,* 2009).

Many virulence factors are associated with the organism's pathogenicity by facilitating adhesion to host cells, leading to infections (Skariyachan et al., 2018). The virulence factors include biofilm formation, pyocyanin, and elastase, with some other factors controlled by a system known as quorum sensing (QS) (Sousa and Pereira 2014). One of the virulence genes is *toxA*, produced by *P. aeruginosa,* which prevents protein synthesis and peptide formation (Nikbin et al*.,* 2012; Fazeli and Momtaz 2014). The presence of the *toxA* gene among *P. aeruginosa* isolates displayed a vital role in the initiation of respiratory symptoms as affected lung and trachea (Tartor and El-Naenaeey, 2016). Exotoxin type A is secreted by the type II secretion system. Also, Quorum Sensing, Biofilm Formation, Type VI Secretion Systems, are major virulence factors acting in different manners in the immune system. (Rosha et al., 2019). Another virulent factor is the *lasB* gene, which has an important role in acute lung infections (Bielecki et al., 2008). the pathogenesis of *P. aeruginosa* is associated with different virulence factors such as *toxA, exo (S, Y, and U), opr (L and I), and las,* which can destroy the host cells (Haghi et al*.,* 2019). The group of *opr genes* is the major element of lipoproteins in the outer membrane of *P. aeruginosa,* which act as indicators to identify the infection caused by *P. aeruginosa* (Nikbin *et al.* 2012).

The QS mechanism regulates the number of relevant virulence factors (Papenfort and Bassler, 2016; Baskan et al., 2021). The system controlled by the *las* and *rhl,* . (Brindhadevia et al., 2020; Sırıken et al*.,* 2020;). QS states to the capability of a bacteria to sense information from other cells when they reach a serious concentration (i.e. a quorum) to regulate the production of different virulence factors (Elnegery et al*.,* 2021). There are different virulence-encoding genes, such as toxin A (*tox*A), alkaline protease (*apr*A), and elastase (*lasB), which* are regulated by the *las* and *rh*l genes (Attiah et al*.,* 2021). Numerous virulence factors may initiate its pathogenicity. Accumulating different virulent factors can cause invasiveness to the bloodstream and tissue damage (Zeb et al*.,* 2017).

This work was conducted to detect the prevalence, and demonstrate the occurrence of six virulent genes (*lasL, lasB toxA, exoU, exoT*, and *oprl* genes) by PCR, serogroups, and antibiotic-resistant mechanism of P*seudomonas aeruginosa* from different chicken farms (broilers and layers).

MATERIALS AND METHODS

Sampling

The birds showed respiratory symptoms like nasal discharges, and difficulty breathing were collected. The cases were from broiler and layers every 10 samples from the same farm were considered as one case. 200 cases represented different Egyptian governorates (Giza, Sharkia, Qualiubia, Damnhour, Fayoum, and Gharbia) in the supplementary Table 1. in the period from May to November 2021.

The birds were slaughtered according to animal ethics and approval of the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP) - Animal Health Research Institute (AHRI). heart blood- hearts- lungs and yolk in the case of one old chick were collected, transported aseptically in sterile plastic bags, and put in an ice box, each bag was labeled (the label had the name of the governorate- farm name - the type of activity- organ name) to bacteriology unit -RLQP AHRI.

Bacteriological isolation and identification

Isolation and biochemical identification of *P. aeruginosa*, buffered peptone water (BPW) as a pre-enrichment media was inoculated at 37ºC for 24 h with 1 g of organ sample, a loopful of BPW was streaked onto Trypticase soy agar (Oxoid) and was incubat-

ed at 37°C for 24-48 h, the suspected colonies were streaked on MacConkey agar (Oxoid) and *Pseudomonas* agar media with CetriNix Supplement (FD029) (Hi media) at 37°C for 24h. to observe the non-lactose fermenting colonies the method was described by with some modification (Shukla and Mishra, 2015).

The identification analysis included (Gram stain, and motility) and some biochemical tests such as oxidase and catalase, Blood agar hemolysis, nitrate reduction tests, and gelatin hydrolysis were done following (Quinn et al., 2002). The identified isolates were stored at -80° with the addition of glycerol until molecular examination.

Serological identification

Polyvalent and monovalent diagnostic antisera were used for serotyping of *Pseudomonas* isolates. They included (4) vials of polyvalent and (16) vials of monovalent antisera as follows (Mast Assure TM *P. aeruginosa.* antisera) according to the manufacturer's protocol (Bio-Rad®, France) and (Glupczynski *et al.,* 2010). Distribution of *P. aeruginosa* into groups based on *P. aeruginosa* O antisera, as described in the International Antigen Typing Scheme (IATS) according to (Legakis *et al* ,1982).

Antimicrobial susceptibility testing:

The isolates were grown on Mueller-Hinton Agar (OXOID)**.** All *Pseudomonas* isolates were tested for various antimicrobial drugs (OXOID), as follows: Ampicillin (AMP;10 µg); Tetracycline (TE;30 µg)), Streptomycin (S;10 µg)), Sulfamethoxazole (SXZ;100 µg)), Erythromycin (E;15 µg)), Naldixic- acid (NA;30 µg)), Ciprofloxacin (CF;5 µg)), and Norfloxacin (NOR;10 µg)). The inhibition zones were measured in millimeters and scored as sensitive, intermediate, and resistant categories following the critical cutoff point that was mentioned in the Clinical and Laboratory Standards Institute. Also, the interpretation of inhibition zones of test culture was according to (CLSI 2013).

Molecular assessment

The cultured broth was submitted for further examination by molecular technique (PCR).

DNA extraction

QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used for DNA purification according to manufacturer instructions with some modifications. Briefly, take 200 µl of cultured isolate added to 10 µl of proteinase K and 200 µl of lysis buffer then incubate at 56°C for 10 min. After that addition of 200 µl of 100% ethanol. then washed and centrifuged. Elution was done by the addition of 60 µl of elution buffer.

PCR amplification

The used Primers were delivered from Metabion (Germany), the used Primers were listed in Table (1).

The PCR reaction was done with a reaction volume 25- µl. briefly, primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.

The products of PCR obtained from the thermal cycler were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm.

Gel analysis

20 µl of the PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) and gelpilot 100 bp plus DNA ladder (Qiagen, gmbh, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the Data was analyzed using Automatic Image Capture Software (Protein Simple, formerly Cell Biosciences, San Jose, CA, USA).

RESULTS

Bacteriological assay

Bacteriological examination revealed 18 positive isolates of *pseudomonas* out of 200 examined cases with a total percentage of (18/200 = 9 %). As shown in Table (2). As the pseudomonas aeruginosa has a characteristic colony on TSA (The basic colony of *P. aeruginosa* is circular, raised have a pigment production pyocyanin (blue-green) the selected colonies were identified on MacConky agar (colorless colonies) and pseudomonas agar plates (blue-green pigmentation) as non-lactose fermenter.

According to biochemical tests, the recovered isolates showed a positive reaction of oxidase and catalase and gave β-hemolysis on blood agar and positive for nitrate reduction test. The eighteen strains under-

went a Serological examination. All the strains revealed *aeruginosa* and the isolates belonged to groups (O1, O4, O6, O11, and O12).

The results of antimicrobial susceptibility testing:

All isolates of the *P. aeruginosa* were resistant (100%) to Sulfamethoxazole, Erythromycin, Tetracycline, and Ampicillin. Also, fifteen isolates showed resistance (83.3%) to Streptomycin and Nalidixic acid. But all isolates exhibited complete sensitivity to Ciprofloxacin. four isolates showed sensitivity with a percentage of (22.2%) to Norfloxacin. As shown in Table (3).

PCR results

The examined isolates were confirmed by PCR by amplification of species-specific 16S RNA gene, all isolates were confirmed as *P. aeruginosa.*

on the examination of some virulent genes, all 18 strains showed positivity for (*lasl, toxA, oprl and exoU* with 100%. The *LasB* gene was present with a percentage 16.7% (3/18) and finally, (4/18) were also positive for *exoT* gene with a percentage of 22.2% as shown in figure (1).

The amplified products for Individual genes were shown in Figures 2 and 7.

Table (1): Targeted genes, oligonucleotide primers, amplicon sizes and references for conventional PCR

Target gene	Primers sequences (5'-3')	Amplified segment (bp)	Reference
toxA	GACAACGCCCTCAGCATCACCAGC	396 bp	**Matar *et al.*, 2002**
	CGCTGGCCCATTCGCTCCAGCGCT		
oprL	ATG GAA ATG CTG AAA TTC GGC	504 bp	**Xu *et al.*, 2004**
	CTT CTT CAG CTC GAC GCG ACG		
lasB	ACAGGTAGAACGCACGGTTG	1220 bp	**Finnan *et al.*, 2004**
	GATCGACGTGTCCAAACTCC		
lasI	ATGATCGTACAAATTGGTCGGC	606 bp	**Bratu *et al.*, 2006**
	GTCATGAAACCGCCAGTCG		
exoT	AATCGCCGTCCAACTGCATGCG	152 bp	**Winstanley *et al.*, 2005**
	TGTTCGCCGAGGTACTGCTC		
exoU	CCGTTGTGGTGCCGTTGAAG	134 bp	
	CCAGATGTTCACCGACTCGC		
16S R-RNA	GGGGGATCTTCGGACCTCA	956 bp	**Spilker *et al.*, 2004**
	TCCTTAGAGTGCCCACCCG		

Table (2): Incidence results for *Pseudomonas* spp. isolated from examined samples:

Age	Types ofsamples	No. of examinedcases	No. of isolates	Percentage according to no of examined cases
One day old chick	Yolk sac	80	4	5%
Broilers	Heart blood and lung	100	11	11%
layers	heart	20	3	15%
Total	---	200	18	9%

Table (3): The antimicrobial resistance results for *P. aeruginosa* isolates.

Antimicrobial agents	Conc.	Resistance No /18 (%)	Intermediate No /18 (%)	Sensitive No /18 (%)
Sulphamethazole (SX)	100µg	18\100	0	0
Erythromycin (E)	15µg	18\100	0	0
Tetracycline (TE)	30 µg	18\100	0	0
Ciprofloxacin (CIP) (CIP)	5 µg	0	0	100%
Ampicillin (AMP)	10 µg	18\100	0	0
Streptomycin (S)	10 µg	15 (83.3)	2 (11.1)	1 (5.6)
Nalidixic acid (NA)	30 µg	15 (83.3)	3(16.7)	0
Norfloxacin (NOR)	10 µg	14 (77.8)	0	4 (22.2)

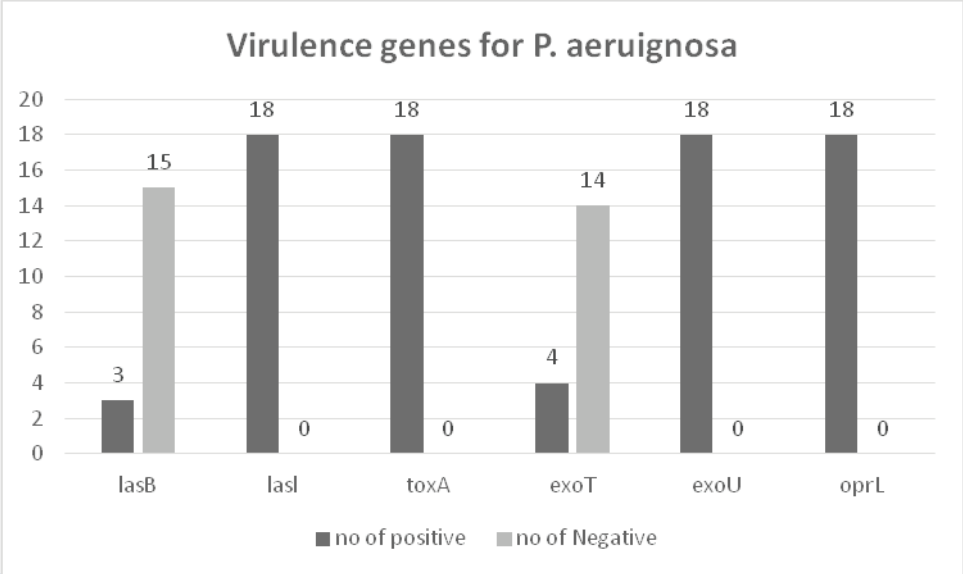

Figure 1 The results of virulent genes for the examined 18 *Pseudomonas aeruginosa* strains by PCR.

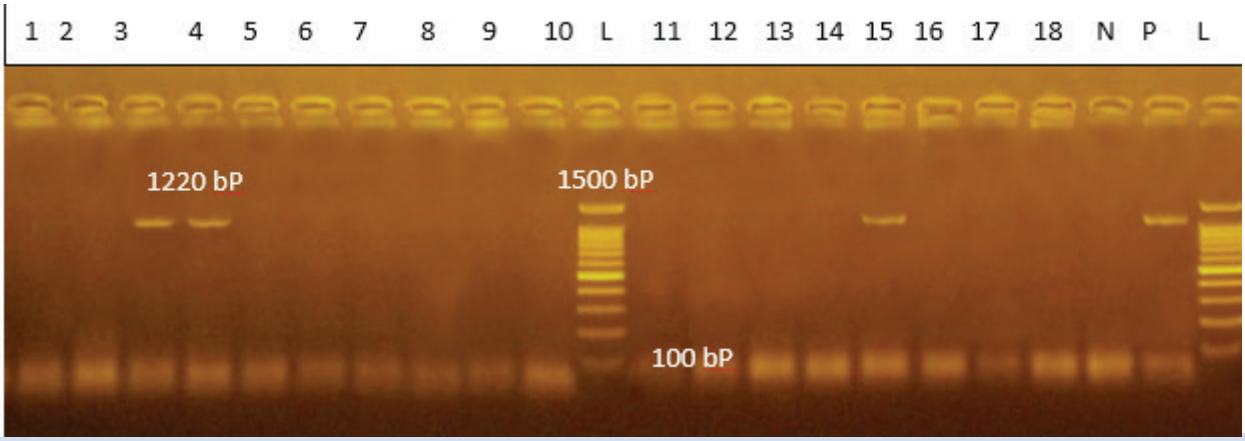

Figure (2): *LasB* gene amplification for *pseudomonas aeruginosa*

The figure showed PCR products for the amplified *LasB* gene for *pseudomonas aeruginosa.* Lane 1: 18 represent examined strains and positive amplification appeared at 1220bp. Lane N represent negative Lane P represent positive reference *P. aeruginosa* control. Lane L represented Gelpilot 100 bp plus Ladder (11 bands).

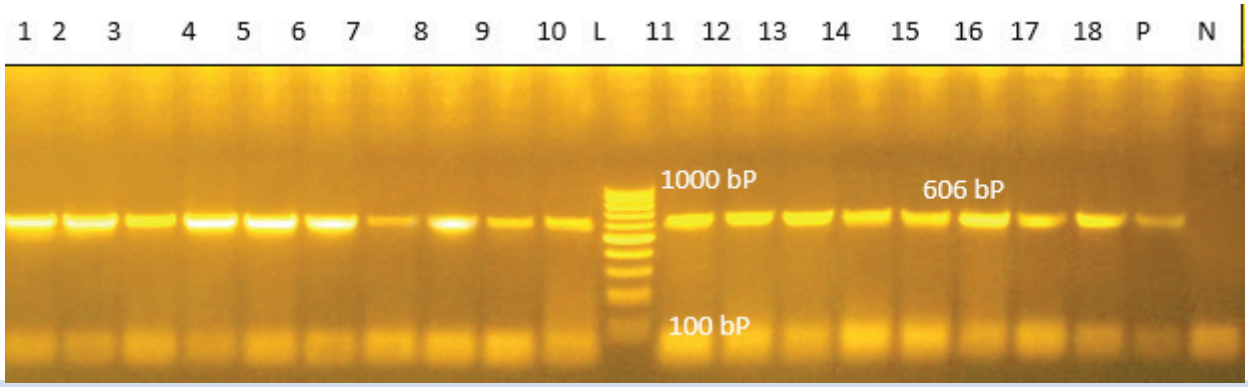

Figure (3): *LasL* gene amplification for *pseudomonas aeruginosa*

The figure showed PCR products for the amplified LasL gene for *pseudomonas aeruginosa*. Lane 1: 18 represent examined strains and positive amplification appeared at 606bp. Lane N represent negative Lane P represent positive reference *P. aeruginosa* control. Lane L represented Gelruler 100 bp Ladder (10 bands).

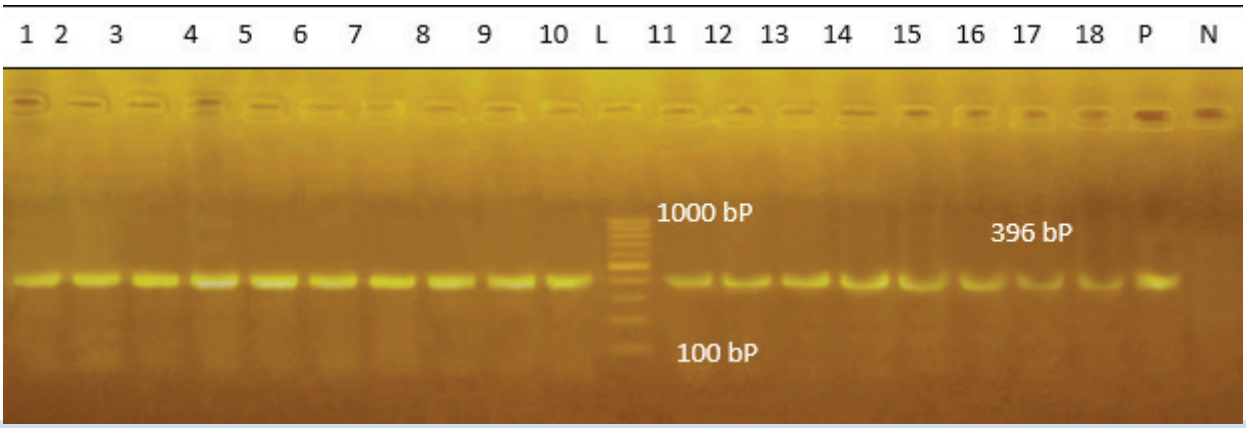

Figure (4): *toxA* gene amplification for *pseudomonas aeruginosa*

The figure showed PCR products for the amplified toxA gene for *pseudomonas aeruginosa.* Lane 1: 18 represent examined strains and positive amplification appeared at 396 bp. Lane N represent negative Lane P represent positive reference *P. aeruginosa* control. Lane L represented Gelruler 100 bp Ladder (10 bands).

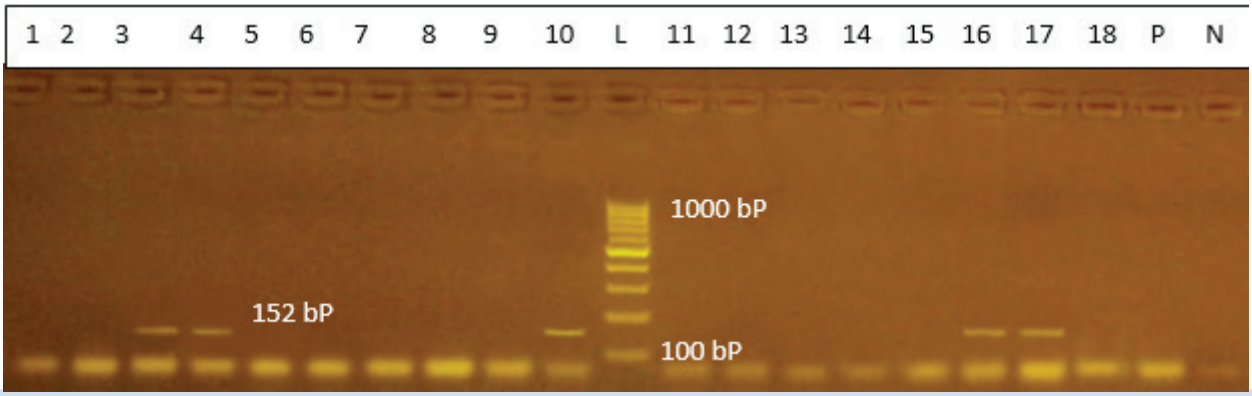

Figure (5): *exoT* gene amplification for *pseudomonas aeruginosa*

The figure showed PCR products for the amplified *exoT* gene for *pseudomonas aeruginosa*. Lane 1: 9 and from lanes 10 to 18 represent examined nine strains and positive amplification appeared at 152 bp. Lane N represent negative Lane P represent positive reference *P. aeruginosa* control. Lane L represented Gelruler 100 bp Ladder (10 bands).

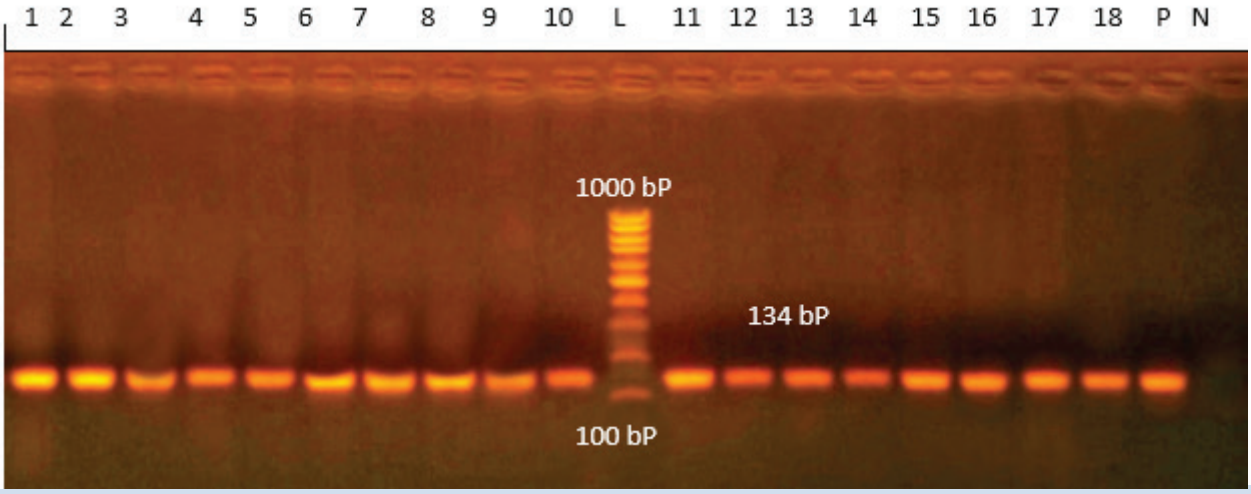

Figure (6): *exoU* gene amplification for *pseudomonas aeruginosa*

The figure showing PCR products for the amplified *exoU* gene for ***pseudomonas aeruginosa***. Lane 1: 18 represent examined strains and positive amplification appeared at 134 bp. Lane N represent negative Lane P represent positive reference *P. aeruginosa* control. Lane L represented Gelruler 100 bp Ladder (10 bands).

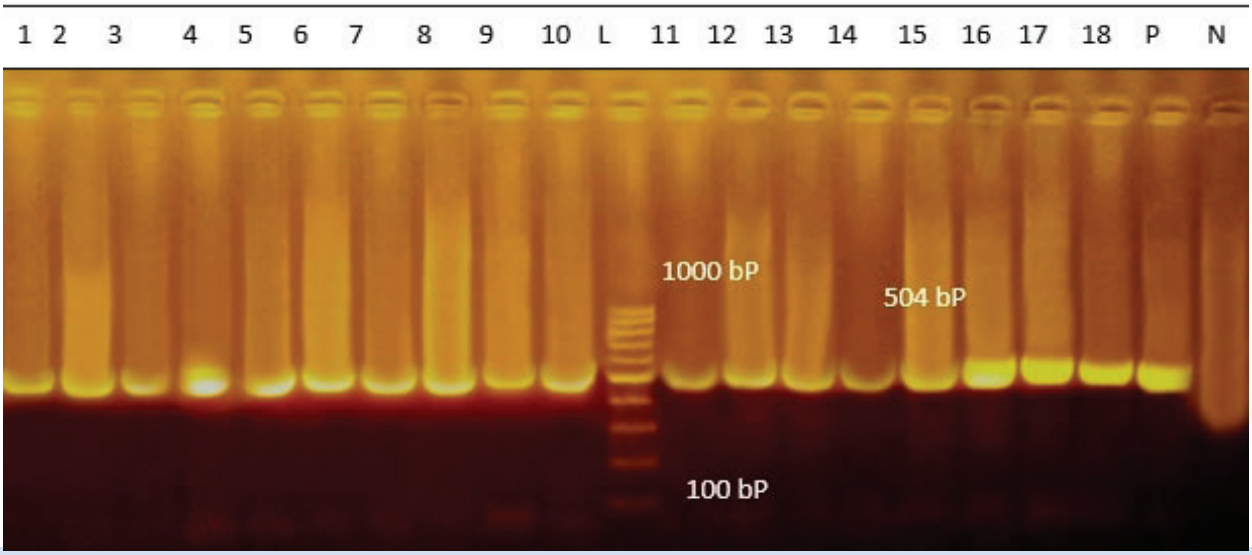

Figure (7): *oprL* gene amplification for *pseudomonas aeruginosa*

The figure showed PCR products for the amplified oprL gene for pseudomonas aeruginosa. Lane 1: 18 represent examined strains and positive amplification appeared at 504 bp. Lane N represent negative Lane P represent positive reference P. aeruginosa control. Lane L represented Gelruler 100 bp Ladder (10 bands).

DISCUSSION

Infection by *Pseudomonas* species in birds is an opportunistic disease that can be transmitted through poultry flocks, producing different lesions in the respiratory system and leading to deaths (Walker *et al.,* 2002). Many surveys have been directed at the incidence of *P. aeruginosa* infection among different bird flocks. In this research, the total incidence of *P. aeruginosa* was 9%. Furthermore, the percentage in broiler and layer flocks was 11% and 15%, respectively some studies as Satish and Priti (2015) identified *P. aeruginosa* with a percentage of 12% in healthy chicks and 30% in diseased ones. Our results were nearly like to those of Mohamed (2004), who found that the percentage of *P. aeruginosa* was 7.6% in broiler chickens isolated from many Egyptian governorates. Furthermore, *P. aeruginosa* has been detected with a percentage of (8.75%) in broiler chicken samples (Farghaly *et al.,* 2017). On the contrary, Hassan *et al*.,2013 recovered *P. aeruginosa* from diseased broilers with an incidence of 25.3% and with a percentage of 10% in dead broilers. In addition, a single percentage of 52% of dead-in-shell embryos was documented by Elsayed et al., 2016. recently, Badr et al., 2020 recorded a high incidence (69.57%) from broiler chicken farms and (39.78%) from chicks. Moreover, Eraky et al., 2020, reported a percentage of 8% (16/200) of *P. aeruginosa* from hatcheries with an incidence of 8.25% (17/206) in samples from chicken embryos. In the present study, antimicrobial susceptibility testing was conducted using eight different antimicrobial agents. The highest recorded results were obtained as the resistance reached (100%) with sulfamethoxazole, erythromycin, tetracycline, and ampicillin which give difficulty for treatment by those types. However, all of the strains were sensitive to ciprofloxacin, and it is considered an effective antibiotic that can be used for P. *aeruginosa infection* treatment, followed by norfloxacin (22.2%). Three strains out of 18 only exhibited sensitivity to nalidixic acid (16.7%), some authors as Hassan *et al,.* 2020 recorded that all the examined isolates were resistant to different numbers of antimicrobial agents (e.g., ampicillin, cefoxitin, lincomycin, sulfamethoxazole, doxycycline, and florfenicol). Also, they concluded that treating *P. aeruginosa* was difficult due to a significant increase in antimicrobial resistance. Moreover, the results in this study agreed with (Eraky *et al.,* 2020) that stated different strains of *P. aeruginosa* showed susceptibility (100%) to ciprofloxacin and 48.48%. streptomycin. In addition, 100% resistance was recorded with other antimicrobials such as erythromycin, amoxicillin, clavulanic acid, and doxycycline. and was 78.78% with nalidixic acid. The strains of *P. aeruginosa* use different resistant mechanisms to overcome the most commonly used antibiotics (Pang *et al.,* 2019). in this study *P. aeruginosa* tested isolates showed a multidrug resistance pattern, this finding was also discussed by (Karp *et al.,* 2017), who found that multidrug-resistant bacteria reflect human risk or veterinary risk. The present study confirmed that *P. aeruginosa* was resistant to multiple antimicrobial drugs and has several

mechanisms to decrease its susceptibility to them as well; it showed this resistance during treatment (Pang *et al,* 2019). Also, our findings agreed with (Sousa and Pereira 2014) who suggested that *P. aeruginosa* produced many virulence factors in a synchronized system to enable host colonization and adaptation. Also (Morita *et al,* 2015) recorded that *P. aeruginosa* has various virulence components, either expressed in coded plasmids or included with other chromosomal genes.

Quorum sensing is bacterial communication through particular chemical elements that regulate their action by constructing virulence factors and antimicrobial resistance mechanisms (Asfour *et al.,* 2018). Some authors revealed that QS system-deficient strains could not create infections due to virulent factors downgrading gene expression (Kumar *et al.,* 2009). Also there are limited literature data about the QS system with the presence of virulent genes and their relation to genetic diversity (Bogiel *et al.,* 2021; El-Mahdy and El-Kannishy, 2019). Virulent genes might contain many proteins used for hydrolysis in host cells, causing a disturbance in the immune system and causing inflammation and damage to the tissue (Haghi *et aal.,* 2019; Everett and Davies, 2021). In this research, all the examined strains of *P. aeruginosa* exhibited *lasl, toxA, exoU,* and *oprL* genes. while three strains and four out from 18 *P. aeruginosa* strains only appeared positive to *lasB* and *exoT,* respectively, as in Figure 1. The *toxA* gene can be used as a marker for identifying strains of *P. aeruginosa* by using a polymerase chain reaction (Auda *et al.,* 2015). in the present research *tox*A, was found in all the examined strains, and these results matched to many previously documented studies (Auda *et al.,* 2015; Elsayed *et al.,* 2016; Morales-Espinosa *et al.,* 2017). also agreed to that recorded by Hassan et al., 2020 who studied different *Pseudomonas spp.* Against diverse virulent genes then confirmed the existence of virulent genes such as *toxA, lasI, and lasB*, in 100%, 80%, and 80%, respectively.

One of the factors regulated and encoded by lasB is elastase, a protease that affects host defense immune-regulatory proteins (Dulon *et al.,* 2004). In this research*, the las*B gene was detected in only three strains out of 18 (3/18= 16.7%), and this incidence differed from that designated by (Benie *et al.* 2017) who detected the prevalence of *lasB* at 80%. The *LasI* gene was responsible for the secretion of some virulence factors, such as pyocyanin and elastase (Bratu *et al.,* 2006).

in the present work, the *lasI* and *oprl* genes were found with high percentage that reached 100%, and these results were nearly agreed with that reported by (Bratu *et al*.,2006) who detected 80%. Of *las*I and also, agreed with (Baskan et al., 2021) that recorded a high prevalence for *oprl*. In our research of isolates associated with pathogenicity and respiratory signs, we found *exo*U-positive isolates to be in all examined strains. This contrasts with previous studies using isolates associated with diverse cases and suggests that *exoU*-mediated cytotoxic activity may be an advantage for P. However, a significant privation of *exo*U suggested that the gene was not essential in P. aeruginosa pathogenicity Lomholt et al. (2001)

CONCLUSION

P. aeruginosa was isolated from suspected diseased cases of chickens (layers and broilers) from different Egyptian governorates providing a conclusion that poultry have the presence of multiple antibiotic resistances between strains in this study, in addition to the dissemination of multi-virulent genes amongst them which increase their resistance to treatment Since *P. aeruginosa* develops a complicated antibiotic resistance strategy, the most effective upcoming treatments will likely require combinational remedies. Also the presence of more than virulent genes so *P. aeruginosa* is considered a serious problem in poultry production and need a respectable program for treatment and or prophylactic doses of effective antimicrobial drugs.

CONFLICT OF INTEREST

None declared

ACKNOWLEDGMENTS

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REFERENCES

Abd El-Ghany WA (2021) *Pseudomonas aeruginosa* infection of avian origin: Zoonosis and one health implications, Vet. World 14(8): 2155-2159.

Attiah SA, Majeed GH, Mohammed TK, (2021) Molecular detection of the *exoU* and *toxA* genes among *Pseudomonas aeruginosa* of patients with burn and wound infection in Baghdad City. Annals R.S.C.B,25(6): 109-122

Asfour HZ (2018) Anti-quorum sensing natural compounds. J Microsc Ultrastruct, 6(1):1-10; https://doi.org/10.4103/JMAU. JMAU_10_18.

Auda IG, Al-Kadmy, IMS, Ali ANM, Muslim SN, Salman IMA (2015) *ToxA* gene as a chromosomal marker for rapid identification of Otitis media.*Pseudomonas aeruginosa*. Trans Sci Techno2 (2):6- 10; https://doi.org/10.15242/IJACEBS.C0815022

Azam MW and Khan AU (2019) Updates on the pathogenicity status of *Pseudomonas aeruginosa* . Drug Discov. Today; 24(1): 350-359.

Badr JM, El Saidy FR and Abdelfattah AA (2020) Emergence of multidrug resistant *Pseudomonas aeruginosa* in broiler chicks. Int. J. Microbiol. Biotechnol.; 5(2): 41-47.

Baskan C, Sinken B, tufekci EF, Klinc- Erturk O, and Erol I (2021) Quorum Sensing Mediated Pathogenicity, Virulence Genes and Class 1 Integron in Carbapenem-Resistant Clinical *Pseudomonas aeruginosa* Isolates.; DOI: https://doi.org/10.21203/rs.3.rs-1148175/v1

Benie CKD, Dadié A, Guessennd N, DésiréKouame N, Nadège A, Kouadio NAN et al (2017) Molecular identification and virulence factors of *Pseudomonas aeruginosa* strains isolated from animal products. J Bacteriol Mycol 2017; 4(3):91-6; https://doi.org/10.15406/ jbmoa. 04.00094.

Bielecki P, Glik J, Kawecki M,VAA MS (2008) Towards understanding *Pseudomonas* aeruginosa burn wound infections by profiling gene expression.; 30; (5):777-90.

Bratu S, Gupta J, Quale J (2006) Expression of the las and rhl quorum-sensing systems in clinical isolates of *Pseudomonas aeruginosa* does not correlate with efflux pump expression or antimicrobial resistance. J Antimicrob Chemother 58(6):1250-3; https://doi.org/10.1093/jac/dkl407.

Bogiel T, Prażyńska M, Kwiecińska- Piróg J, Mikucka A, Gospodarek-Komkowska E. Carbapenem M (2021) Resistant *Pseudomonas aeruginosa* strains distribution of the essential enzymatic virulence factors genes. Antibiotics (Basel), 10(8): 1-10. doi: 10.3390/antibiotics10010008

Brindhadevia K, Oscarb FL, Mylonakisb E, Shanmugamc S, Vermad TN et al (2020) Biofilm and Quorum sensing mediated pathogenicity in *Pseudomonas aeruginosa*. Process Biochem.; 96: 49-57. doi: 10.1016/j.procbio.2020.06.001.

Chen SH, Fegan N, Kocharunchitt C, Bowman JP and Duffy LL (2020) Changes of the bacterial community diversity on chicken carcasses through an Australian poultry processing line. Food Microbiol., 86: 103350.

CLSI: Clinical and Laboratory Standards Institute (2013) M100-S23 performance standards for antimicrobial, Wayne, PA.

Dulon S, Leduc D, Cottrell GS, D'Alayer J, Hansen KK, Bunnett NW et al (2005) *Pseudomonas aeruginosa* elastase disables proteinase-activated receptor 2 in respiratory epithelial cells. Am J Respir Cell Mol Biol; 32(5):411-9; https://doi.org/10.1165/rcmb.2004-0274OC

El-Mahdy R, El-Kannishy G (2019) Virulence factors of carbapenem-resistan*t Pseudomonas* aeruginosa in hospital-acquired infections in Mansoura, Egypt. Infect Drug Resist.12: 3455-3461. doi: 10.2147/IDR.S222329

Elnegery AA, Mowafy VK, Zahra TA, El-Khier NTA (2021) Study of quorum-sensing LasR and RhlR genes andtheir dependent virulence factors in *Pseudomonas aeruginosa* isolates from infected burn wounds. Access Microbiol. 3(3): 1-11. doi: 10.1099/acmi.0.000211.

Elsayed MSA, Ammar AM, Al-Shehri ZS, Abd-El Rahman H and Abd-El Rahman NA (2016) Virulence repertoire of *Pseudomonas aeruginosa* from some poultry farms with detection of resistance to various antimicrobials and plant extracts. Cell. Mol. Biol., 62: 124-128.

Eraky RD, Abd El-Ghany WA and Soliman KM (2020) Studies on *Pseudomonas aeruginosa* infection in hatcheries and chicken. J. Hellenic Vet. Med. Soci. 71(1):1953-1962.

Everett MJ and Davies DT (2021) *Pseudomonas aeruginosa* elastase (*LasB*) as a therapeutic target. Drug Discov Today. 26(9): 2108-2123.

Farghaly EM, Roshdy H, Bakheet AA, Abd El-Hafez SA and Badr H (2017) Advanced studies on Pseudomonas aeruginosa infection in chicken. Anim.Health Res. J., 5(4): 207-217.

Fazeli N and Momtaz H (2014) Virulence gene profiles of multidrug-resistant *Pseudomonas aeruginosa* isolated from Iranian hospital infections, Iranian Red Crescent Medical Journal, vol. 16 (10), p. e15722.

Finnan S, Morrissey JP, Gara FO, Boyd EF (2004) Genome Diversity of *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients and the Hospital Environment.Journal of clinical microbiology, 42(12): 5783-92.

Glupczynski Y, Bogaerts P, Deplano A, Berhin C, Huang C, Van J, Villalobos H (2010) Detection and characterization of class A extended-spectrum b-lactamase-producing *Pseudomonas aeruginosa* isolates in Belgian hospitals. Journal of antimicrobial agents and Chemotherapy, 65: 866-871.

Gong Q, Ruan MD, Niu MF, Qin CL, Hou Y and Guo JZ (2018) Immune efficacy of DNA vaccines based on oprL and oprF genes of *Pseudomonas aeruginosa* in chickens. Poult. Sci., 97(12): 4219-4227.

Haghi F, Nezhad BB, Zeighami H (2019) Effect of subinhibitory concentrations of imipenem and piperacillin on *Pseudomonas aeruginosa toxA* and *exoS* transcriptional expression. New Microbes and New Infect; 32: 1-5. doi:10.1016/j.nmni.2019.100608

Hassan HM (2013) Characterization of *Pseudomonas aeruginosa* Isolated from Different Pathological Lesions in Chickens. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Beni- Suef University, Egypt.

Hassan WH, Ibrahim AMK, Shany SAS (2020) Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt, J. Adv. Vet. Anim. Res.,; 7(3): 452-463.

Karp BE, Tate H, Plumblee JR, Dessai U, Whichard, JM, Thacker E.L, et al. (2017) National antimicrobial resistance monitoring system: two decades of advancing public health through integrated surveillance of antimicrobial resistance. Foodborne Pathog Dis.14(10):545-57; https://doi.org/10.1089/fpd.2017.2283

Kumar R, Chhibber S, Harjai K (2009) Quorum sensing is necessary for the virulence of *Pseudomonas aeruginosa* during urinary tract infection. Kidney Int.; 76(3): 286-92. doi: 10.1038/ki.2009.183.

Legakis N, Majtan V, Wang S (1982) Serotypes of *Pseudomonas aeruginosa* in clinical specimens in relation to antibiotic susceptibility. Journal of Clinical. Microbiology.;16 (3): 458-463.

Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22: 582-610.

Lomholt JA, Poulsen K and Kilian M (2001) Epidemic population structure of Pseudomonas aeruginosa: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. Infect Immun 69, 6284-6295. Mahenthiralingam, E., Campbell, M. E.

Matar GM, Ramlawi F, Hijazi N, Khneisser I, Abdelnoor AM (2002) Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. Current microbiology.; 45: 350-354.

Mohamed HA (2004) Some studies on *Pseudomonas* species in chicken embryos and broiler in Assiut governorate. Ass. Univ. Bull. Environ.; (1): 23-31.

Morita Y, Tomida J, Kawamura Y (2015) Efflux-mediated fluoroquinolone resistance in the multidrug-resistant *Pseudomonas aeruginosa* clinical isolate PA7: identification of a novel MexS variant involved in upregulation of the mexEF -oprN multi-drug efflux operon. Front Microbiol 6:8; https://doi.org/10.3389/fmicb.2015.00008

Morales-Espinosa R, Delgado G, Espinosa LF, Isselo D, Mendez JL, Rodriguez C, et al. (2017) Fingerprint analysis and identification of strains ST309 as a potential high risk clone in a *P seudomonas aeruginosa* population isolated from children with bacteremia in Mexico City. Front Microbiol ; 8:313; https://doi.org/10.3389/fmicb.2017.00313.

Nikbin VS Aslani MM Sharafi Z, Hashemipour M, Shahcheraghi F, and Ebrahimipour GH (2012) "Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins," Iranian Journal of Microbiology, vol. 4, no. 3,pp. 118-123.

Pang Z, Raudonis R, Glick BR, Tong-Jun Lina T, and Chenga Z (2019) Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies *Biotechnology* Advances; 37: 177-192

Papenfort K, Bassler BL, (2016) Quorumsensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol .14:576-588.

Quinn PJ, Markey BK, Carter ME, Donnelly WJ and Leonard F (2002): Veterinary Microbiology and Shahat et al., 2019 SVU-IJVS, 2 (2): 52-70 69 Microbial Disease. Iowa State University Press, Ames, and USA.pp.124- 126.

Satish S, and Priti M. (2015) *Pseudomonas aeruginosa* infection in broiler chicks in Jabalpur. Int. J. Ext. Res., 6: 37-39.

Shukla S and Mishra P (2015) *Pseudomonas aeruginosa* infection in Broiler Chicks in Jabalpur, international journal of extensive Research.; 6: 37-39.

Skariyachan S, Sridhar VS, Packirisamy S, Kumargowda ST, Challapilli SB (2018) Recent perspectives on the molecular basis of biofilm formation by *Pseudomonas aeruginosa* and approaches for treatment and biofilm dispersal. Folia Microbiologica. 63:413-432

Sousa A and Pereira M (2014) Pseudomonas aeruginosa diversification during infection development in cystic fibrosis lungs—a review. Pathogens, 3:680-703.

Spilker T, Coenye T, Vandamme P, Lipuma JJ (2004) PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. Journal of Clinical. Microbiology; 42: 2074-2079.

Sırıken B, Öz V, Erol İ (2020) Quorum sensing systems, related virulence factors, and biofilm formation in *Pseudomonas aeruginosa* isolated from fish. Arch Microbiol; 203: 1519- 1528.

Tartor YH and El-Naenaeey EY (2016) RT-PCR detection of exotoxin genes expression in multi- drug resistant *Pseudomonas aeruginosa*. Cell Mol Biol (Noisy-le-grand); 62(1):56-62.

Ven -Heir E, Moen B, Åsli AW, Sunde M and Langsrud S (2021) Antibiotic Resistance and Phylogeny of *Pseudomonas* spp. Isolated over Three Decades from Chicken Meat in the Norwegian Food Chain, Microorganisms; 9, 207.

Walker SE, Sander JE, Cline JL, Helton JS (2002) Characterization of *Pseudomonas aeruginosa* is olates associated with mortality in broiler chicks. Avian Dis; 46(4):1045-50; https://doi.org/10.1637/0005-2086(2002)046[1045:COPAIA]2.0.CO;2

Winstanley C, Kaye SB, Neal TJ, Chilton HJ, Miksch S, Hart CA (2005) Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. Journal of Medical Microbiology.; 54:519-526.

Xu J, Moore JE, Murphy PG, Millar BC, Elborn JS (2004) Early detection of *Pseudomonas aeruginosa*; comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Annals of Clinical Microbiology and Antimicrobials.; 3(1): 21.

Zeb A, Ullah I, Rehman H et al. (2017) Antibiotic susceptibility pattrens of *Pseudomonas aeruginosa* in tertiary care hospital, Journal of Entomology and Zoology Studies, vol. 5, no. 1, pp. 437-439.

Supplementary Table 1.

Governorate	Types of farms			Total no of cases
	Broilers	layers	One day chicks	Per Gov.
Giza	8	2	5	15
Qualiubia	14	2	18	34
Sharkia	24	4	21	49
Gharbia	18	3	14	35
Damnhour	22	5	12	49
Fayoum	14	4	10	28
Total	100	20	80	200`

*the total is calculated according to the total examined farms of all types