Molecular Characterization and Genetic Analysis of *Pseudomonas Aeruginosa* Recovered from Broiler Chickens

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Abstract:

Pseudomonas aeruginosa is an opportunistic pathogen causes serious problems in broiler farms and this pathogen has an economical importance in broiler sector. A total of 200 broiler chickens of different ages [84 broiler chicks (1-7 days) and 116 broiler chickens (1-6 weeks)] were collected from different broilers farms for isolation and identification of *P. aeruginosa*. Different samples were taken from internal organs (liver, gall bladder, lung, heart blood, intestine, kidney, yolk sacs) as well as cloacal swabs. The samples were examined bacteriologically, 28 isolates (14%) of *P. aeruginosa* were isolated and identified by biochemical reactions as well as API 20E system. The yolk sac and cloacal swabs samples gave the highest recovery rates with percentages of 15.5% and 12.6%, respectively. PCR assay confirmed the existence of *P. aeruginosa* DNA in ten isolates by using 16S rRNA gene. Also, PCR assay was carried out to detect the presence of virulence genes as *opr*L, *tox*A and *apr*A. The *opr*L gene was present with a percentage of 100%, *tox*A was present with a percentage of 80%, and *apr*A gene with a percentage of 40%. Also, sequencing of 16S rRNA gene of *P. aeruginosa* was submitted to Gen Bank with accession number MW051028 which was 100% identical to *P. aeruginosa* strains.

Keywords: *P. aeruginosa*, chickens, virulence genes, PCR, 16S rRNA gene sequence.

Introduction:

Pseudomonas species found everywhere and are isolated from various sources such as drinking water, domestic and wild animals, human beings, plants, and from a variety of foods. These Gram-negative bacteria are non-fermentative rods, aerobic, oxidase-positive

and motile with polar flagella ***(Arnaut-Rollier et al., 1999; Jay et al., 2005; Franzetti and Scarpellini, 2007*** **and** ***Adams and Moss, 2008)***. Pseudomonas infections of birds are of a great importance as epidemics may spread rapidly through the poultry flocks causing mortality in all ages ***(Satish and Priti, 2015)***. *P. aeruginosa* is an opportunistic pathogen capable of infecting all tissues ***(Van Delden and Iglewski, 1998*** and ***Khattab et al., 2015)***. It is always classified as the head most of three frequent Gram-negative pathogens and is associated with the worst visual diseases. Its outbreak varies from 2 to 100% ***(Fick, 1993; Barnes, 1997*** and ***Saad et al., 2017)***. *P. aeruginosa* has a variety of virulence factors that frustrate host defenses and make direct tissue's harm or rise bacterium's affordability. The most important virulence factors of *P. aeruginosa* includes Exotoxin A (*eta*), outer membrane associated protein L and I and quorum-sensing determinant system ***(Gellatly and Hancock, 2013)***. Exotoxin A (*eta*) is a strong extracellular virulence factor produced by most of clinical isolates of *P. aeruginosa* ***(Salman, 2015)***. The *apr*A is one of the most important virulence factors of *P. aeruginosa* which is a zinc-dependent metalloprotease ***(Hoge et al., 2010)***. Polymerase chain reaction (PCR) is one of the notable methods for detection and identification of *P. aeruginosa* ***(Deschaght et al., 2011)***. It has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism that is outer lipoprotein membrane (*opr*L) gene in *P. aeruginosa* isolates ***(Khan and Cerniglia, 1994)***. Sequencing of 16S rDNA gene is specifically important in the case of bacteria with unusual phenotypic profiles, rare bacteria, slow growing bacteria, uncultivable bacteria and culture-negative infections. Not only has it provided insights into etiologies of infectious disease, but it also helps in choosing antibiotics and in determining the duration of treatment and infection control procedures ***(Woo et al., 2008)***. Identification of bacteria at species level, DNA sequences at genus-specific might be widely used. Therefore, the present study was planned for bacteriological characterization of *P. aeruginosa* isolated from broiler chickens with different ages, detection of virulence genes as well as sequencing of 16S rRNA gene and submission into Gen Bank.

Material and Methods:

Samples collection

A total of 200 chickens of different ages [84 broiler chicks

(1-7 days) and 116 broiler chickens (1-6 weeks)] were collected from different broilers farms in Suez Canal area for isolation and identification of *P. aeruginosa*. The samples were collected from 51 apparent healthy broiler chickens, 84 diseased broiler chickens showing (profuse diarrhea and respiratory manifestations including coughing, sneezing, rolling, nasal discharges) and 65 freshly dead broiler chickens as shown in **Table (1)**. The samples were collected from internal organs: 200 for each (liver, gall bladder, lung, heart blood, intestine, kidney), 135 cloacal swabs and 84 yolk sacs of chicks. All samples were handled aseptically to prevent cross-contamination using sterile sampling materials and transferred immediately in ice box to the laboratory.

Isolation and Identification

The samples were inoculated in nutrient broth and incubated aerobically for 24hrs at 37ºC. Then a loopful of the inoculated nutrient broth was streaked onto selective media (Pseudomonas agar base) and incubated aerobically for 24hrs at 37ºC. The suspected colonies were inoculated for 24hrs at 37°C onto nutrient agar, sheep blood agar and MacConkey agar. The characteristic colonies of *P. aeruginosa* were selected and the Gram staining was performed. The colonies showing typical colonial appearance were subjected to biochemical identification and examined for Catalase, Oxidase, Urea hydrolysis, Indole, Methyl red, Citrate utilization, Voges-Proskauer and Hydrogen Sulphide production tests described by ***Quinn et al. (2002)***. Also, API 20E (Bio-Mérieux®, France) was used as a confirmatory biochemical test.

Molecular detection of 16S rRNA gene, virulence genes as *opr*L, *tox*A, *apr*A of *P. aeruginosa* isolates by PCR

Conventional PCR assay for confirmation of *P. aeruginosa* isolates and detection of virulence genes was performed. DNA was extracted from the isolated *P. aeruginosa* using QIAamp DNA mini kit (Germany). PCR Master Mix and cycling conditions of the primers during PCR were prepared according to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit. Oligonucleotide primers used in PCR have specific sequence and amplify a specific product as shown in **Table (2)**. DNA samples were amplified in a total of 25µl as follows: 12.5 µl of Emerald Amp GT PCR Master Mix, 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of template DNA. The reaction was performed in a Biometra thermal cycler (Bio-

Rad S1000 USA). Temperature and time conditions of the primers during PCR were applied **(Table, 2)**. Aliquots of amplified PCR products were electrophoresed in 1.5% agarose gel (ABgene) in 1x TBE buffer at room temperature. For gel analysis, 15µl of PCR products were loaded in each gel slot. A 100 bp DNA Ladder (QIAGEN Inc, Valencia, CA, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Gene Sequencing

PCR products were purified using QIA quick PCR Product extraction kit (QIAGEN Inc, Valencia, CA, and USA). Big dye Terminator v3.1 cycle sequencing kit. (Perkin-Elmer/Applied Biosystems, Foster City, CA) was used for the sequence reaction and then it was purified using Centrisep (spin column). DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan) and a comparative analysis of sequences was performed using CLUSTAL W. multiple sequence alignment program, version 1.83 of Meg Align module of Laser gene DNA Star software.

Statistical analysis

The Chi-square test and P value were performed to analyze the obtained results (SPSS software, version 22) (significance level; $P < 0.05$) according to ***Greenwood and Nikulin (1996)***.

Table (1): *Number and type of examined samples*

Source of sample	Apparent healthy	Diseased	Freshly dead	Total
Broiler chicks (1-7 days)	12	44	28	84
Broiler chickens (1-6 weeks)	39	40	37	116
Total	51	84	65	200

Table (2): *Oligonucleotide primers for 16S rRNA and virulence genes of P. aeruginosa, the size of amplified products, and cycling conditions required for detecting the tested genes*

Target gene	Primer sequence (5'-3')	Amplicon size (bp)	Cycling conditions (All genes involved initial denaturation at 95˚C for 2 min. followed by 35 cycles)	Reference
16S rRNA	GGGGGATCTTCGG ACCTCA TCCTTAGAGTGCC CACCCG	956	Denaturation: 94˚C/20 sec; Annealing: 58˚C/20 sec; Extension: 72˚C/40 sec; Final extension: 72˚C/1 min	***Spilker et al., 2004***
***opr*L**	ATG GAA ATG CTG AAA TTC GGC CTT CTT CAG CTC GAC GCG ACG	504	Denaturation: 96˚C/1 min; Annealing: 55˚C/1 min; Extension: 72˚C/1 min; Final extension: 72˚C/10 min	***Xu et al., 2004***
***tox*A**	GGAGCGCAACTAT CCCACT TGGTAGCCGACGA ACACATA	150	Denaturation: 95˚C/40 sec; Annealing: 50˚C/1 min; Extension: 72˚C/2 min; Final extension: 72˚C/10 min	***Sabharwal et al., 2014***
***apr*A**	GTCGACCAGGCGG CGGAGCAGATA GCCGAGGCCGCCG TAGAGGATGTC	993	Denaturation: 95˚C/40 sec; Annealing: 65˚C/1 min; Extension: 72˚C/2 min; Final extension: 72˚C/10 min	

Results:

Morphological and biochemical identification of *P. aeruginosa* isolates

The colonies of *P. aeruginosa* appeared variable in size with blue–green or brown pigment on Pseudomonas agar base medium. On nutrient agar medium, the colonies were large, flat, spreading and irregular with grayish green or brown pigment on the culture with fruity grape–like odour. On blood agar medium, the colonies were surrounded by a wide zone of complete hemolysis (Beta hemolysis). On MacConkey agar medium, large pale colonies were seen with greenish or brown pigment. *P. aeruginosa* isolates appeared to be Gram negative straight or slightly curved rods occurred singly, in pairs or in short chains, non-sporulated and non-capsulated. All isolates showed similar pattern of reaction despite of the source of sampling, it showed positive reaction to Catalase, Oxidase, Citrate and Urea hydrolysis tests while, negative reaction to Methyl red, Voges-Proskauer, Indole and H_2S

production tests. Results of API 20E revealed the probability for *P. aeruginosa* as shown in **Fig. (1)**.

Prevalence of *P. aeruginosa* in broiler chickens

P. aeruginosa was recovered in 28 out of 200 examined chickens with an incidence of 14%. *P. aeruginosa* was isolated from apparently healthy chickens with a rate of 2 (3.9%), diseased 20 (23. 8%) and freshly dead 6 (9.2%). Statistically, there is non-significant difference among the prevalence of *P. aeruginosa* in healthy, diseased and dead chickens. The total number of *P. aeruginosa* isolated from 84 broiler chicks (1-7 days) were 19 with an incidence of 22.6%, and the total number of P. *aeruginosa* isolated from 116 broiler chickens (1-6 weeks) were 9 with an incidence of 7.8% as shown in **Table (3)**. Statistically, there is a significant difference among the prevalence of *P. aeruginosa* in broiler chickens while broiler chicks show non-significant. According to the site of sampling, *P. aeruginosa* was isolated as the following: 4.5% (9 out of 200) from liver; 0.5% (1 out of 200) from lung; 1.5% (3 out of 200) from heart blood; 2.5% (5 out of 200) from intestine; 15.5% (13 out of 84) from yolk sac; 12.6% (17 out of 135) from cloacal swab, and zero from gall bladder and kidney. Statistically, there is a significant difference among the prevalence of *P. aeruginosa* according to the site of sampling.

Molecular confirmation of *P. aeruginosa* DNA by using 16S rRNA gene

Ten *P. aeruginosa* isolates were subjected for molecular examination. PCR results confirmed the existence of *P. aeruginosa* DNA in the ten isolates by using 16S rRNA gene at 956 bp with a total percentage of 100% as shown in **Fig. (2)**.

Molecular detection of virulence genes in *P. aeruginosa* isolates by PCR

All ten *P. aeruginosa* isolates confirmed by 16S rRNA were tested for detection of virulence genes. The results showed that *opr*L gene was detected in all *P. aeruginosa* isolates with a percentage of 100% **(Fig. 3)**. Also *tox*A was detected with the percentage 80% **(Fig. 4)**. While *apr*A gene was detected with a percentage of 40% **(Fig. 5)**.

Sequence analysis of 16S rRNA gene of *P. aeruginosa*

The 16S rRNA gene sequence in this study had the accession number (MW051028) in GenBank. The nucleotide sequence analysis of (956 bp) PCR product representing the 16S rRNA gene of *P. aeruginosa* isolate in this study revealed identity (100%) to various strains of poultry origin as *P. aeruginosa* S-04 16S rRNA gene (MT626658.1) (South Africa);

plant origin as *P. aeruginosa* 23S rRNA gene (MT544458.1) (India), *P. aeruginosa* AM02 16S rRNA gene (MT598016.1) (Indonesia), *P. aeruginosa* AM14 16S rRNA gene (MT598018.1) (Indonesia), *P. aeruginosa* AJ18 16S rRNA gene (MT598021.1) (Indonesia), *P.aeruginosa* AP04 16SrRNA gene (MT598022.1) (Indonesia), *P.aeruginosa* AP17 16SrRNA gene (MT598024.1) (Indonesia) and *P. aeruginosa* AB18 16S rRNA gene (MT598026.1) (Indonesia); different origins as *P. aeruginosa* OIS 4.8.1 16S rRNA gene (MT633047.1) (Russia), *P. aeruginosa* MLTBM2 16S rRNA gene (MT646431.1) (Republic of the Congo), *P. aeruginosa* NC110620 16S rRNA gene (MT623377.1) (India) and *P. aeruginosa* A-7 16S rRNA gene (MT573199.1) (China).

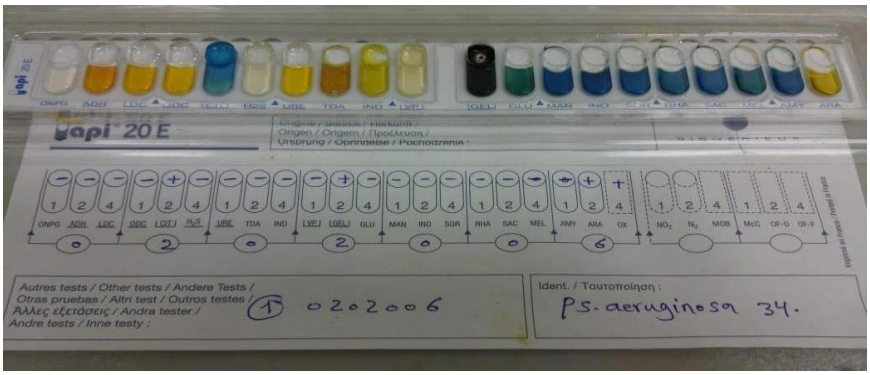

Fig. (1): *Results of API 20E of P. aeruginosa*

Table (3): *Prevalence of P. aeruginosa in examined chickens*

Source of sample	Apparent healthy	%	Diseased	%	Freshly dead	%	Total	%	P value
Broiler chicks (1-7 days)	2	16.7	13	29.5	4	14.3	19	22.6	0.004422
Broiler chickens (1-6 weeks)	-	-	7	17.5	2	5.4	9	7.8	0.01312
Total	2	3.9	20	23.8	6	9.2	28	14	
P value	0.1573		0.1797		0.4142				

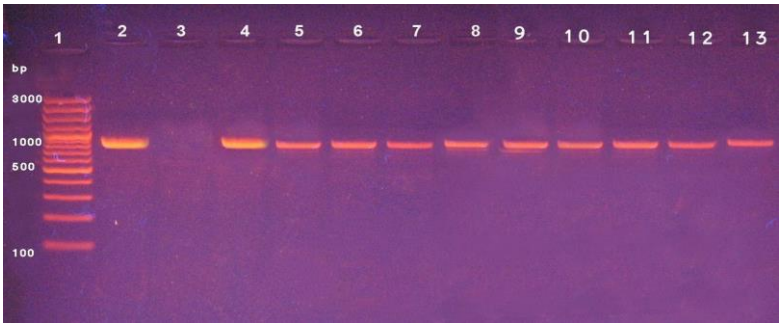

Fig. (2): *Agarose gel electrophoresis showing the result of PCR for detection of 16S rRNA gene (956 bp) from P. aeruginosa strains* ***Lane 1:*** *100bp DNA ladder,* ***Lane 2:*** *Control Positive,* ***Lane 3:*** *Control Negative and* ***Lane 4-13:*** *Positive P. aeruginosa isolates for 16S rRNA gene.*

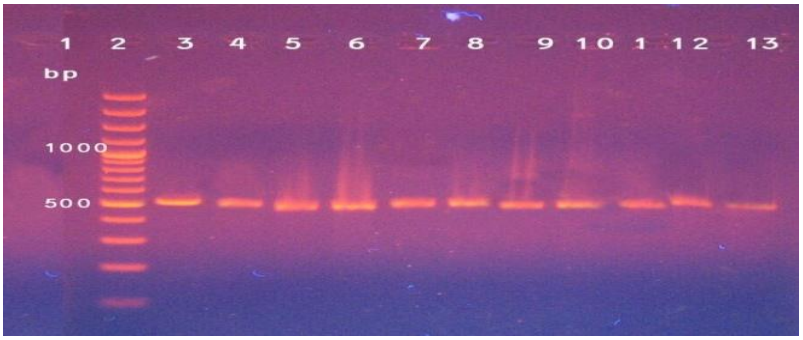

Fig. (3): *Agarose gel electrophoresis showing the result of PCR for detection of oprL gene (504 bp) from P. aeruginosa strains* ***Lane 1:*** *Control Negative,* ***Lane 2:*** *100bp DNA ladder,* ***Lane 3-12:*** *Positive P. aeruginosa isolates for oprL gene and* ***Lane 13:*** *Control Positive.*

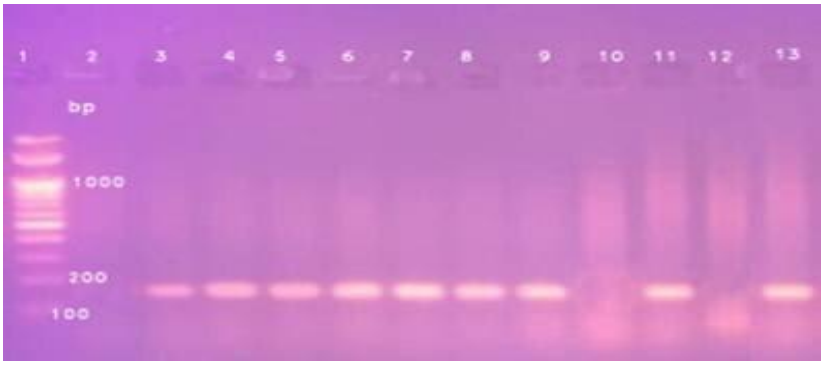

Fig. (4): *Agarose gel electrophoresis showing the result of PCR for detection of toxA gene (150 bp) from P. aeruginosa strains*

Lane 1: *100bp DNA ladder,* ***Lane 2:*** *Control Negative,* ***Lane 3:*** *Control Positive,* ***Lane 4, 5, 6, 7, 8, 9, 11 & 13:*** *Positive P. aeruginosa isolates for toxA gene and* ***Lane 10 & 12:*** *Negative P. aeruginosa isolates for toxA gene.*

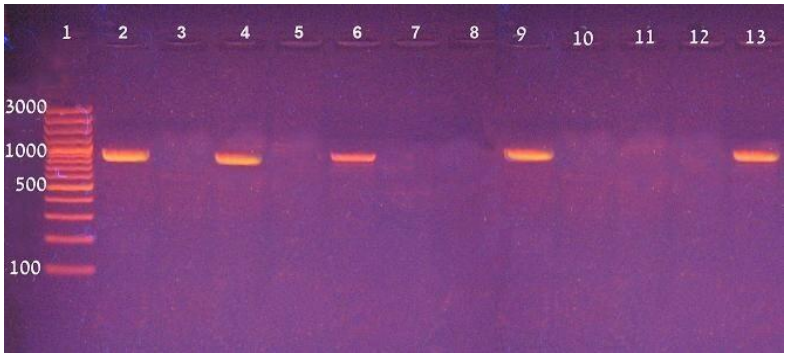

Fig. (5): *Agarose gel electrophoresis showing the result of PCR for detection of aprA gene (993 bp) from P. aeruginosa strains*
Lane 1: *100bp DNA ladder, Lane* ***2:*** *Control Positive, Lane* ***3:*** *Control*
Negative, ***Lane 4, 6, 9 & 13:*** *Positive P. aeruginosa isolates for aprA gene and* ***Lane 5, 7, 8, 10, 11 & 12:*** *Negative P. aeruginosa isolates for aprA gene.*

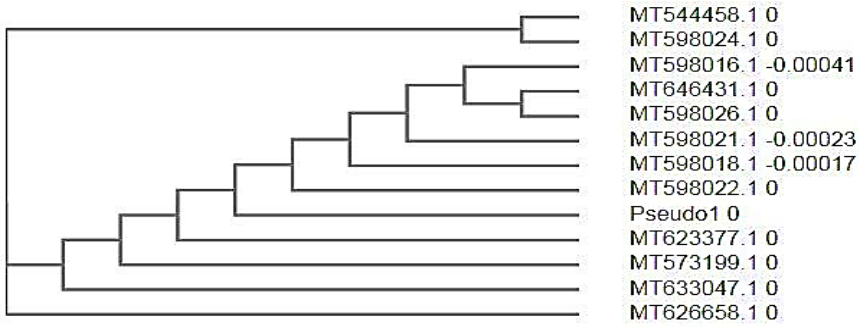

Fig. (6): *The phylogenetic analysis of 16S rRNA gene of P. aeruginosa*

Discussion:

P. aeruginosa is a problem of economic concern to poultry industry. Therefore, this study tries to illustrate the problem of *P. aeruginosa* in chickens with screening of virulence genes, as well as sequencing that aid in epidemiological relatedness. In this study, 200 broiler chickens of different ages collected from different broilers farms in Suez Canal area were examined for the presence of *P.*

aeruginosa. The results illustrated in **Table (3)** showed that the incidence of *P. aeruginosa* in the examined chickens was 14% (28 out of 200). These results are nearly similar to those recorded by ***Sidhom (2007)*** who found that *P. aeruginosa* was recovered from chickens with an incidence of 13.34% and ***Enany et al. (1986) and Omran (2012)*** who recovered *P. aeruginosa* from chickens with an incidence of 12%. In contrast to the present study, lower incidence rates were recorded by ***Awaad et al. (1981)*** who isolated *P. aeruginosa* with an incidence of 2.9% from an outbreak in a broiler flock, and ***Abd El- Tawab et al. (2014)*** who isolated 17 isolates of *P. aeruginosa* with an incidence of 4.57%. While, higher incidence rate was recorded by ***Abd El-Tawab et al. (2018)*** who isolated *P. aeruginosa* with a percentage of 34%. Concerning the incidence of *P. aeruginosa* in broiler chickens and chicks, results obtained in this study showed that the higher incidence rate was recorded in broiler chicks (1-7 days) 22.6%, in comparison with old ages (1-6 weeks) 7.8% as shown in **Table (3)**, these results were supported by ***Kebede (2010)*** who proved experimentally that the main cause of high mortalities in unhatched chicken and young chicks, was the infection by *P. aeruginosa* in hatching time. These results are nearly agreeing with those recorded by ***Mohamed (2004)*** who isolated *P. aeruginosa* from 85 baby chicks (2-15 days) and 60 broilers (5-8 weeks) with rates of 17.6% and 3.3% respectively, ***Farghaly et al. (2017)*** who recorded that *P. aeruginosa* isolated from chickens was with a percentage of 5.7% (16/280) and from one–day old chicks 13% (26/200). On the other hand, these results are not consistent with ***Hassan (2013)*** who isolated *P. aeruginosa* from chickens with an incidence of 25.3% and from one-day-old chicks with an incidence of 8%, ***Abd El-Tawab et al. (2014)*** who isolated *P. aeruginosa* from broiler chickens (1-5weeks) with a percentage of 4.85%, meanwhile one isolate of *P. aeruginosa* was isolated from one day old chicks with a percentage of 2.38%. The difference in the prevalence rates may be due to socio-economic factors, climatic changes, health status of birds, food and water supply variation with geographical situation. This study demonstrated also that diseased chickens showed a higher rate with an incidence of 23.8%, in comparison with freshly dead and apparently healthy chickens with incidence rates of 9.2% and 3.9%,

respectively as shown in **Table (3)**. These results are nearly similar to those recorded by ***Farhan (2006)*** who isolated *P. aeruginosa* from diseased chickens with an incidence of 20.5%, ***Satish and Priti (2015)*** who isolated *P. aeruginosa* from clinically diseased with a rate of 30%. While, ***Abd El- Tawab et al. (2014)*** reported that the incidence rates of *P. aeruginosa* between diseased, freshly dead and apparently healthy chickens were 6.25%, 2.38% and 1.56%, respectively. Concerning the incidence of *P. aeruginosa* according to the site of sampling, *P. aeruginosa* was isolated from liver, intestine, heart blood, lung, yolk sac and cloacal swabs samples but it was not isolated from neither gall bladder nor kidney samples. The yolk sac and cloacal swabs samples gave the highest recovery rates with percentages of 15.5% and12.6%, respectively. These results are nearly similar to those recorded by ***Abd El-Tawab et al. (2014)*** who reported that cloacal swabs samples gave the highest recovery rate with an incidence of 18.3%. PCR method has been used to provide a specific, rapid, simple, and vastly sensitive discovery of *P. aeruginosa* ***(Qin et al., 2003)***. In this study, PCR assured the presence of *P. aeruginosa* DNA in ten isolates with a percentage of 100% by using specific primers for *P. aeruginosa* (16S rRNA) at 956bp as shown in **Fig. (2)**, these findings were backed by ***Spilker et al. (2004)*** who reported that 16S rDNA-based PCR assays provided rapid, simple, and reliable identification of *P. aeruginosa* and its differentiation from other phylogenetically closely related Pseudomonas species and it show 100% sensitivity and specificity. The results obtained in this study are in corroboration with ***Shahat et al. (2019)*** who used PCR to inveterate the existence of *P. aeruginosa* DNA in the all seven isolates (100%) by using 16S rDNA primers at 956 bp. Also, ***Abdel-Tawab et al. (2016)*** revealed that *P. aeruginosa* (16S rDNA) gene amplification of 956 bp was observed in the extracted DNA of three isolates out of five tested isolates. However, ***Al-Dahmoshi et al. (2018)*** confirmed *P. aeruginosa* using specific primer for 16S rDNA gene of *P. aeruginosa* at 956bp with a percentage of 22.8%. Our study was designed for the detection of virulence genes as *opr*L, *tox*A and *apr*A in *P. aeruginosa* isolates by using PCR. The *opr*L gene is an outer membrane lipoprotein of *P. aeruginosa* which is responsible for inherent resistance of *P. aeruginosa* to antibiotics and antiseptics. As this protein is found only in this organism, it

could be a reliable factor for rapid identification of *P. aeruginosa* in clinical samples ***(Osayande, 2008*** and ***Douraghi et al., 2014)***. It was found that all ten *P. aeruginosa* isolates showed positive amplification of 504 bp fragment specific for *opr*L gene (common gene) with a total percentage of 100% as shown in **Fig. (3)**, it confirmed the existence of *P. aeruginosa* DNA because it considers a specific marker for molecular detection of *P. aeruginosa*. These results are the same obtained by ***Abd El-Tawab et al. (2014)*** who used PCR for detection of *opr*L gene of *P. aeruginosa* in six isolates and all isolates gave a positive amplification of 504 bp fragment specific for the *opr*L gene of *P. aeruginosa* (species-specific gene), also, ***Elsayed et al. (2016); Shahat et al. (2019)*** and ***Bakheet and Torra (2020)*** recorded that *opr*L gene was disclosed in all *P. aeruginosa* isolates with a percentage of 100%. The *tox*A gene, an inherent genetic sequence located on *P. aeruginosa* chromosome and regulating the synthesis of exotoxin A (*exo*A), has been widely used as a target for *P. aeruginosa* detection in PCR methods ***(Khan and Cerniglia, 1994; Qin et al., 2003*** and ***Xu et al., 2004)***. The *tox*A gene was reported in the present study with a percentage of 80% as shown in **Fig. (4)**, these results were supported by ***Vasil et al. (1986)*** and ***Xu et al. (2004)*** who stated that the *tox*A gene is produced by the majority of *P. aeruginosa* strains. These results agree with ***Farghaly et al. (2017)*** and ***Bakheet and Torra (2020)*** who recorded high distribution of *tox*A gene among *P. aeruginosa* isolates with rates of 80.9% and 83.3%, respectively. Higher prevalence of *tox*A gene in *P. aeruginosa* strains (100%) was recorded by ***Sabharwal et al. (2014)***. Also, ***Abd El-Tawab et al. (2018); Elmouaden et al. (2019); Shaha et al. (2019); Qian et al. (2020)*** and ***Al-Dahmoshi et al. (2018)*** recorded that *tox*A (*exo*A) gene was disclosed in *P. aeruginosa* isolates with percentages of 95%, 74.2%, 71.4%, 60% and 46.15%, respectively. The *apr*A gene is an important virulence gene and contributes towards the pathogenicity of *P. aeruginosa*. It was present in four isolates (4 out of 10) with a percentage of 40% as shown in **Fig. (5)**. This result is in between those recorded by ***Sabharwal et al. (2014)*** who recorded that *apr*A gene was present in a percentage of 16.6% in *P. aeruginosa* isolates, and ***Qian et al. (2020)*** who recorded that the prevalence of *apr*A gene in *P. aeruginosa* strains was 75%. The analysis of phylogenetic tree revealed identity (100%) of 16S rRNA

gene of *P. aeruginosa* isolate in this study to various strains of plant, poultry and other various origins ***(Dutta et al., 2019*** and ***Maulidia et al., 2021)***. This epidemiological relatedness revealed that plants may be considered a source of infection.

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توصيف جزيئي وتحليل جيني للسودوموناس ارجينوزا المعزولة من دجاج التسمين

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الملخص العربى

يعتبر السودوموناس ارجينوزا ممرض انتهازي و يسبب مشاكل خطيره فى مزارع التسمين وذا أهميه اقتصاديه في قطاع الدواجن. تم تجميع عدد ٢٠٠ دجاجة تسمين من الأعمار المختلفة [عدد ٨٤ كتكوت تسمين (١- ٧ يوم)، عدد ١١٦ دجاجة تسمين (١- ٦ أسبوع)] من مزارع تسمين مختلفة لعزل ميكروب السودوموناس ارجينوزا. وتم تجميع عينات مختلفة من الأعضاء الداخلية (الكبد، المرارة، الرئه، دم القلب، الأمعاء، الكلى، الكيس المحى) بالأضافه الي مسحات الزرق. تم فحص العينات بكتريولوجيا، وتم عزل ٢٨ معزول من السودوموناس ارجينوزا بنسبة ١٤٪ وتم التعرف عليها بواسطة التفاعلات البيوكيميائيه و API 20E، وأظهرت عينات الكيس المحى ومسحات من الزرق أعلى معدل عزل بنسب ١٥,٥٪ و ١٢,٦٪ على التوالى. لقد أكد اختبار تفاعل البلمرة المتسلسل تواجد الحمض النووى الخاص بالسودوموناس ارجينوزا فى العشرة معزولات المفحوصة باستخدام جين 16S rRNA كما تم اجراء اختبار تفاعل البلمرة المتسلسل للكشف عن بعض جينات الضراوه مثل*opr*L و *tox*A و *apr*A وكانت النتائج كالتالى: تواجد جين *opr*L فى جميع المعزولات (١٠٠٪)، تواجد جين*tox*A بنسبة ٨٠٪ ، بينما تواجد جين *apr*A بنسبة ٤٠٪, وأيضا تم اجراء التتابع الجينى لجين 16S rRNA للسودوموناس ارجينوزا وتسجيلها ببنك الجينات برقم MW051028 وكانت نسبة التطابق مع معزولات السودوموناس ارجينوزا تصل الى ١٠٠٪ .