

ORIGINAL ARTICLE

## Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt

Walid Hamdy Hassan<sup>1</sup>, Ahmed Mohamed Kamel Ibrahim<sup>2</sup>, Salama Abohamra Sayed Shany<sup>3</sup>, Hala Sayed Hassan Salam<sup>1</sup>

<sup>1</sup>Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt

<sup>2</sup>Beni-Suef University Hostel, Beni-Suef University, Beni-Suef, Egypt

<sup>3</sup>Department of Poultry Diseases, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt

### ABSTRACT

**Objectives:** This study was performed to probe the antimicrobial resistance and virulence genes profiling in *Pseudomonas aeruginosa* recovered from the cases of pericarditis in broiler chickens.

**Materials and Methods:** The samples ( $n = 250$ ) collected from the cases of pericarditis in broiler chickens were bacteriologically examined. Antimicrobial susceptibility was tested by disc diffusion technique. The isolates were genotypically studied for the presence of antimicrobial resistance and virulence gene traits. Finally, the nucleotide sequence of representative resistance gene (*mexR* gene) and virulence genes (*toxA* and *lasI* genes) was analyzed.

**Results:** *P. aeruginosa* was isolated from 45 samples (18%). Antimicrobial susceptibility testing revealed multidrug resistance in most of the recovered *P. aeruginosa* isolates, whereas colistin and imipenem were the furthestmost *in vitro*-sensitive antibiotics. Antimicrobial resistance genes, such as *bla*<sub>CTX</sub>, *fox*, and *mexR*, were prevalent in 100%, 80%, and 100% of the isolates, respectively. PCR confirmed virulence genes such as *toxA*, *exoY*, *lasB*, and *lasI* in 100%, 60%, 80%, and 80% of the isolates, respectively. Nucleotide sequence analysis of representative resistance gene (*mexR* gene) and virulence genes (*toxA* and *lasI* genes) revealed a high correlation between *P. aeruginosa* recovered from pericarditis in broiler chickens in the present study with PAO1 (reference strain) and with other sequences published on the GenBank representing different localities worldwide.

**Conclusion:** It could be concluded that *P. aeruginosa* recovered from pericarditis in broiler chickens in the current study is highly virulent bacteria, resisting most of the therapeutic agents which not only bear hazards for poultry industry but also represent a public health concern.

### ARTICLE HISTORY

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### KEYWORDS

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### Introduction

Poultry farms are wide open to many bacterial species infections resulting in the different clinical sequel, which affects the investments in the poultry industry badly thus, decreasing the total amount of produced protein and/or transmitting pathogens of zoonotic importance to human [1,2]. *Pseudomonas* infection in birds is an essential opportunistic bacterial disease which may spread promptly through poultry flocks, triggering mortality rates and producing different and often distinctive postmortem lesion [3].

*Pseudomonas aeruginosa* has numerous virulence elements, either encoded on plasmids or chromosomal

genes [4]. Exotoxin A (ETA) and elastase are of the major virulence factors of *P. aeruginosa* and are of great significance; ETA is an important ADP-ribosyltransferase toxin that encoded by *toxA* gene [5,6]. Elastase is a protease that can reduce host defense, immunoregulatory proteins, and impaired epithelia, which encoded by *lasB* gene [7]. *LasI* gene (auto-inducer) is responsible for the secretion of some virulence tools of *P. aeruginosa* as elastase and pyocyanin [8]. Exotoxin Y is a type III secretion system effector that is a dual soluble adenylyl and guanylyl cyclase, resulting in intracellular cAMP and cGMP accumulation and the creation of interendothelial cell gaps and increased macromolecular permeability [9].

**Correspondence** Hala Sayed Hassan Salam ✉ [hala\\_saydh@yahoo.com](mailto:hala_saydh@yahoo.com) 📧 Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt.

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Antimicrobial agents are needed to treat different types of diseases and to save healthy and productive birds, and antimicrobial agents used in livestock production have been measured as a risk aspect in the evolution and spreading of drug resistance in livestock production farms [10]. Food-producing animals and their surrounding environments act as a reservoir for both resistant bacteria and resistance genes that could be indeed transmitted to humans either by direct contact between humans and animals or indirectly through the food production chain [11].

Antimicrobial resistance is of significance in poultry bacterial infections as it may change treatable diseases into untreatable ones and act as an essential factor for the transmission of resistance to human flora or other bacterial pathogens. Beta-lactams are a significant antibiotic group used to treat infections of humans and animals and to prevent diseases [12]. Their uses have always been followed by the development of resistance, most commonly caused by beta-lactamases [13]. Extended-spectrum beta-lactamases and plasmid-mediated AmpC beta-lactamase are important causes behind the antimicrobial resistance [14]. Therefore, this study was aimed to investigate the antimicrobial resistance, virulence, and resistance genes of *P. aeruginosa* isolated from pericarditis in diseased broiler chickens.

## Materials and Methods

### Sample collection

Pericardial sac and heart blood specimens were collected aseptically according to the OIE protocol for a sample collection from 250 slaughtered diseased and freshly dead broiler cases of 1–5 weeks old (each one broiler chicken represented one farm) in Beni-Suef and Fayoum Governorate, Egypt, in the period from January to June 2018. These chickens were suffered from respiratory manifestations and/or signs of septicemia.

### Isolation and biochemical identification of *P. aeruginosa*

Nutrient broth, nutrient agar, and cetrinide agar medium were used for primary isolation and further cultivation. The identification analysis such as Gram stain, motility, oxidase, catalase, pigment production, growth at 42°C, hemolysis on blood agar, gelatin hydrolysis, and nitrate reduction tests was done as per the standard techniques [15]. The identified isolates were stored at –70° in 20% glycerol stocks until studied.

### Antimicrobial susceptibility testing

The antibiogram of identified isolates was tested by Kirby–Bauer disc diffusion technique against 22 antimicrobial agents comprising 11 different antimicrobial classes of both human and veterinary significance. The suspensions of the

isolates equivalent to McFarland turbidity standard tube No. 0.5 were prepared, and the Mueller–Hinton agar plates were inoculated. Antimicrobial discs (oxid) of amikacin (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), apramycin (15 µg), aztreonam (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), colistin (10 µg), doxycycline HCL (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), gentamicin (10 µg), imipenem (10 µg), lincomycin (10 µg), piperacillin (100 µg), polymyxin-B (300 IU), spectinomycin (100 µg), spiramycin (100 µg), and sulfamethoxazole-trimethoprim (23.75 + 1.25) were applied on the plates. The plates were then incubated at 37°C for 24 hours, and the detected inhibition zones were analyzed according to the guidelines of the Clinical and Laboratory Standards Institute [16]. Multidrug-resistant isolates (MDR) were determined by resistance to at least one agent in three or more antimicrobials of different tested classes [17].

### Identification of virulence and resistance genes

Polymerase chain reaction (PCR) was performed for the recognition of four virulence (*toxA*, *exoY*, *lasB*, and *lasI*) and three resistance (*bla<sub>ctx</sub>*, *fox*, and *mexR*) genes. The extraction of genomic DNA by QIAamp DNA Extraction Miniprep Kit from confirmed cultures is done according to the manufacturer's instructions. Extracted DNA was kept at –80°C till used in PCR amplification. Positive control DNA was obtained from confirmed positive *P. aeruginosa* field isolate in RLQP (Reference laboratory for veterinary quality control on poultry production, Dokki, Giza, Egypt). On the contrary, a negative control is a PCR mixture free from the DNA template. The sequences of primers, sizes of amplified segments, and their references are mentioned in Table 1. PCR conditions were conducted as described previously by listed authors.

### Gene sequencing and sequence analysis

The amplified products of *mexR*, *lasI*, and *toxA* genes were purified from the gel using QIAquick Gel Extraction Kits (Qiagen). All steps of purification were carried following the manufacturer's instructions using reagents provided in the kit and then sequenced directly using the ABI Prism 3,100 automated sequencing machine (Applied Biosystems, Foster City, CA), and a nucleotide BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>) was carried for each sequence. Sequence comparisons and maximum likelihood phylogeny testing through a bootstrap of 1,000 trials were determined with MEGA X software [18] after the Clustal W alignment algorithm. Nucleotide and amino acid identities were determined using Geneious®7.1.3, Build 2014-03-17, Java Version 1.7, Copyright® 2005–2014 Biomatters Ltd.

**Table 1.** Oligonucleotide primers sequences for different antimicrobial resistance and virulence genes of *P. aeruginosa*.

Target gene	Primers direction	Primers sequences (5'-3')	Amplified product size (bp)	Reference
<i>bla<sub>CTX</sub></i>	F	ATGTGCAGYACCAGTAARGTKATGGC	593	[39]
	R	TGGGTRAARTARGTSACCAGAAAYCAGCGG		
<i>Fox</i>	F	CAAAGCGCGTAACCGGATTGG	190	[40]
	R	CAAAGCGCGTAACCGGATTGG		
<i>mexR</i>	F	GCGCCATGGCCATATTCCAGG	637	[41]
	R	GCATTGCCAGTAAGCGG		
<i>toxA</i>	F	GACAACGCCCTCAGCATCACCAGC	396	[42]
	R	CGCTGGCCATTGCTCCAGCGCT		
<i>exoY</i>	F	CGGATTCTATGGCAGGGAGG	289	[43]
	R	GCCCTTGATGCACTCGACCA		
<i>lasB</i>	F	ACAGGTAGAACGCACGGTTG	1,220	[44]
	R	GATCGACGTGTCCAAACTCC		
<i>lasI</i>	F	ATGATCGTACAAATTGGTCGGC	606	[8]
	R	GTCATGAAACCGCCAGTCG		

F = forward; R = reverse; bp = base pair.

## Results

### Isolation of *P. aeruginosa* from broiler chickens

Bacteriological examination revealed that of 250 examined cases, 45 were positive for *P. aeruginosa* isolation with a percentage of 18%.

### Antimicrobial susceptibility testing of *P. aeruginosa* isolates

The results shown in Table 2 revealed that the most sensitive antimicrobials were colistin and imipenem (100% each), amikacin (92%), ceftazidime (92%), polymyxin-B (77.7%), gentamicin (75.5%), cefepime (71.1%), and piperacillin (68.9%). In comparison, there was a lower degree of sensitivity to enrofloxacin (53.3%), aztreonam (44.4%), and spectinomycin (33.3%). On the other hand, all tested isolates were resistant (100%) to ampicillin, doxycycline, cefoxitin, florfenicol, lincomycin, and sulfamethoxazole-trimethoprim, followed by 95.5% and 91.2% of the isolates which were resistant to erythromycin and cefotaxime, respectively. All *P. aeruginosa* isolates under test showed MDR pattern as they resisted at least one member of three different classes and extended to 14 of the tested antimicrobial classes within five (11.11%) of the tested isolates.

### Distribution of antimicrobial resistance genes

All the tested isolates (100%) were positive to *bla<sub>CTX</sub>* and *mexR*, whereas 36 of 45 isolates (80%) were positive to *fox* gene (Figs. 1–3).

### Distribution of virulence genes

All tested isolates harbored *toxA* gene, whereas, out of them, 36 carried both *lasB* and *lasI* genes with 80%, and 27 isolates (60%) confirmed positive to *exoY* gene (Figs. 4–7).

### Sequence analysis

The sequences of representative amplicons of *toxA*, *mexR*, and *lasI* genes have been deposited to the GenBank and have approved GenBank accession numbers MN450176: MN450178; respectively. Amino acid and nucleotide sequence analysis (as shown in Tables 3–5) revealed a high correlation most of the time with other *P. aeruginosa* sequences published on the GenBank representing different clinical lesions, hosts, and localities worldwide, especially with *P. aeruginosa* reference strain (PAO1). Phylogenetic trees (Figs. 8–10) explained the correlation between the sequences of the current study with other sequences.

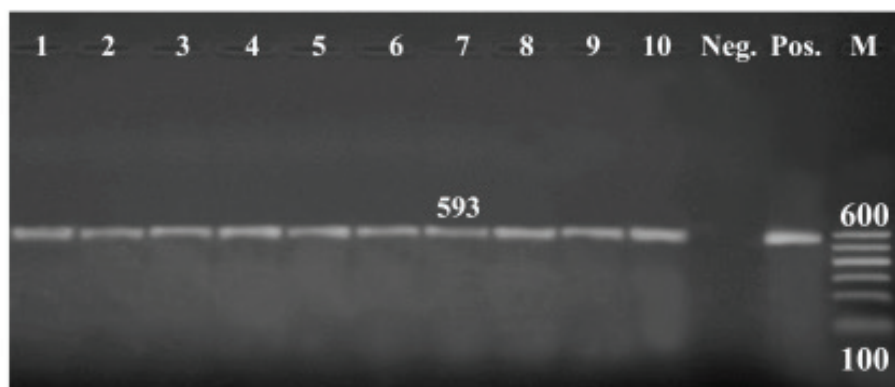
## Discussion

In this study, trials were made to examine 250 specimens from the pericardial sac of broiler chickens with lesions of pericarditis for the isolation of *P. aeruginosa*. Forty-five isolates were obtained out of the studied cases with an occurrence of 18%. This prevalence was relatively higher than that obtained by Abed [19], who recovered 15 isolates of *P. aeruginosa* with a prevalence of 5% from the pericardial sac of broiler chickens with pericarditis and [20] who

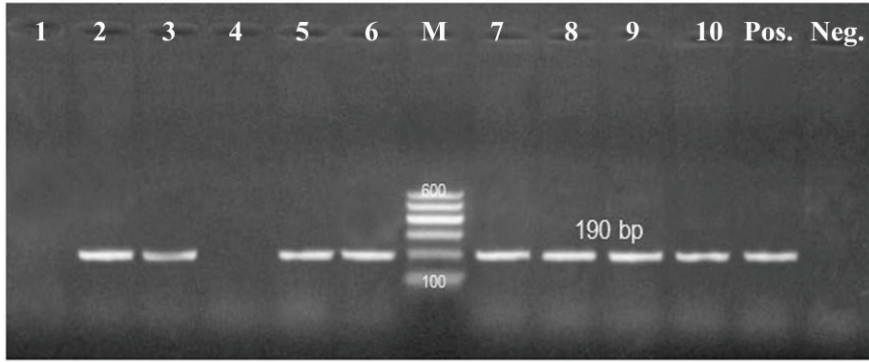
**Table 2.** Antimicrobial susceptibility of *P. aeruginosa* isolated from broiler chicken.

Antimicrobial group	Antimicrobial Agents	Disk content (µg/disk)	<i>P. aeruginosa</i> (n = 45)					
			Susceptible		Intermediate		Resistant	
			No.	%	No.	%	No.	%
β-Lactams (including combination)	Ampicillin	10	0	0	0	0.0	45	100
	Amoxicillin + Clavulanic acid	30	20	44.4	0	0	25	55.6
	Pipracillin	100	31	68.9	6	13.4	8	17.7
	Cefepime	30	32	71.1	2	4.4	11	24.4
	Cefotaxime (ω)	30	0	0.0	4	8.8	41	91.2
	Cefoxitin (n)	30	0	0.0	0	0.0	45	100
	Ceftazidime (ω)	30	41	92	3	6.6	1	2.2
	Carbapenems (Imipenem)	10	45	100	0	0	0	0
Aminoglycosides	Amikacin	30	41	92	2	4.4	2	4.4
	Apramycin	15	4	8.8	23	51.2	18	40
	Gentamicin	10	34	75.5	9	20	2	4.5
	Spiramycin	100	5	11.1	9	20	31	68.9
Polypeptides	Polymyxin- B	300-U	35	77.7	0	0	10	22.3
	Colistin sulphate	10	45	100	0	0	0	0
Monobactam	Aztreonam	30	2	4.4	23	51.1	20	44.4
Fluoroquinolones	Enrofloxacin	5	21	46.7	0	0	24	53.3
Lincosamides	Lincomycin	10	0	0.0	0	0	45	100
Macrolides	Erythromycin	15	2	4.5	0	0	43	95.5
Phenicol	Florofenicol	30	0	0	0	0	45	100
Aminocyclitol	Spectinomycin	100	15	33.3	16	35.5	14	31.2
Tetracycline	Doxycycline HCL	30	0	0.0	0	0.0	45	100
Sulfonamides	Sulfamethoxazole-trimethoprim	23.75 + 1.25	0	0	0	0	45	100

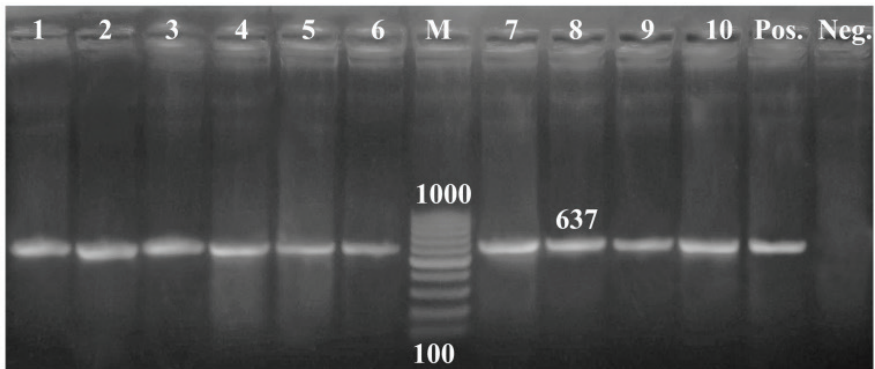
% = percentage was calculated in relation to the total number of isolates.



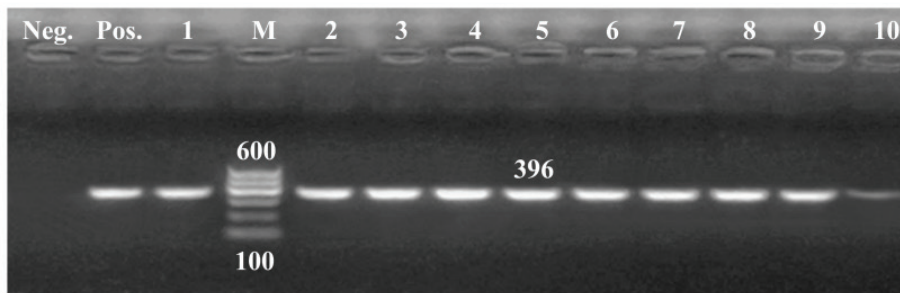
**Figure 1.** PCR amplification of the *bla*CTX gene at 593-bp. Lane (1–10): Showed +Ve isolates. M = DNA ladder; Pos = Control positive; Neg = Control negative.



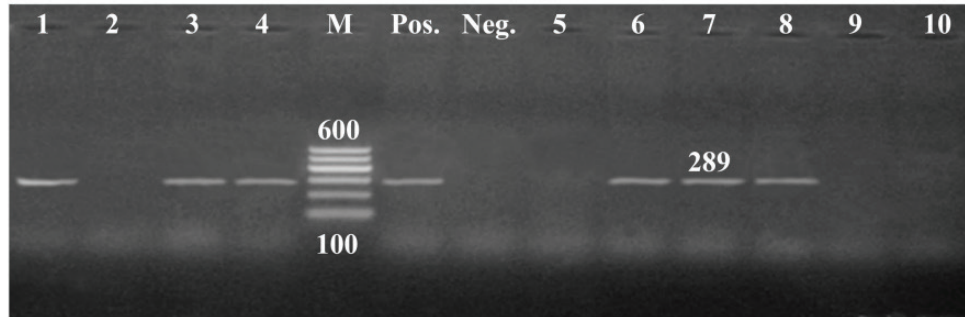
**Figure 2.** PCR amplification of the *fox* gene at 190-bp. Lane(2,3,5,6,7,8,9,10): Showed +Ve isolates, Lane (1,4): Showd -Ve isolates, M = DNA ladder; Pos = Control positive; Neg = Control negative.



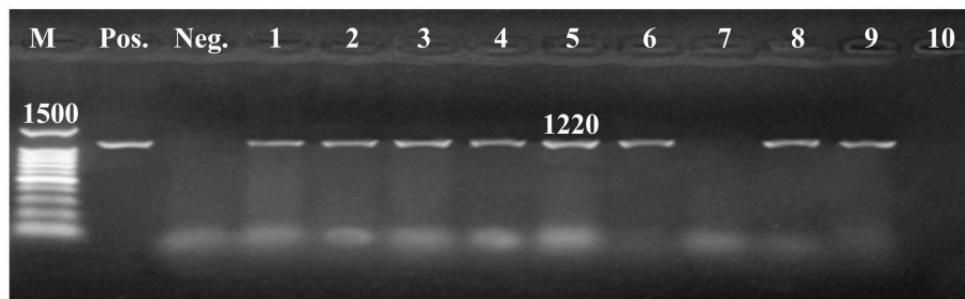
**Figure 3.** PCR amplification of the *mexR* gene at 637-bp. Lane (1-10): Showed +Ve isolates. M = 100 bp DNA ladder; Pos = Control positive; Neg = Control negative.



**Figure 4.** PCR amplification of *toxA* gene of 396pb fragment. Lane (1-10): Showed +Ve isolates. M = 100 bp DNA ladder; Pos = Control positive; Neg = Control negative.



**Figure 5.** PCR amplification of *exoY* gene of 289bp fragment. Lane (1,3,4,6,7,8): Showed +Ve isolates, Lane (2,5,9,10): Showed -Ve isolates, M = 100 bp DNA ladder; Pos = Control positive; Neg = Control negative.



**Figure 6.** PCR amplification of *lasB* gene of 1220 bp fragment, Lane (1,2,3,4,5,6,8,9): Showed +Ve isolates, Lane (7,10) : Showed -Ve isolates, M = DNA ladder; Pos = Control positive; Neg = Control negative.



**Figure 7.** PCR amplification of *lasI* gene of 606bp fragment. Lane (1,2,3,4,5,6,7,9): Showed +Ve isolates, Lane (8,10): Showed -Ve isolates, M = 100 bp DNA ladder; Pos = Control positive; Neg = Control negative.

recovered seven isolates of *P. aeruginosa* with a prevalence of 3.5% from the heart blood of broiler chickens with congestion and pericarditis.

*An in vitro* susceptibility testing was applied against 22 antimicrobial agents, and the most effective antibiotics were colistin sulfate and imipenem (100%), amikacin and ceftazidime (92%), followed by polymyxin-B (77.7%), gentamicin (75.5%), and cefepime (71.1%). Gill et al. [21]

mentioned that colistin is a bactericidal antibiotic well thought out as a critical option for treating infections with MDR-resistant *P. aeruginosa*. The high sensitivity of the studied isolates to imipenem agreed with that of Viedma et al. [22]. Carbapenems, especially meropenem and imipenem, are a crucial group of  $\beta$ -lactams used for the treatment of pseudomonas infections due to their stability to most  $\beta$ -lactamases. The observed sensitivity of the tested

**Table 3.** Nucleotides and amino acids identity of *toxA* gene sequences of *P. aeruginosa*.

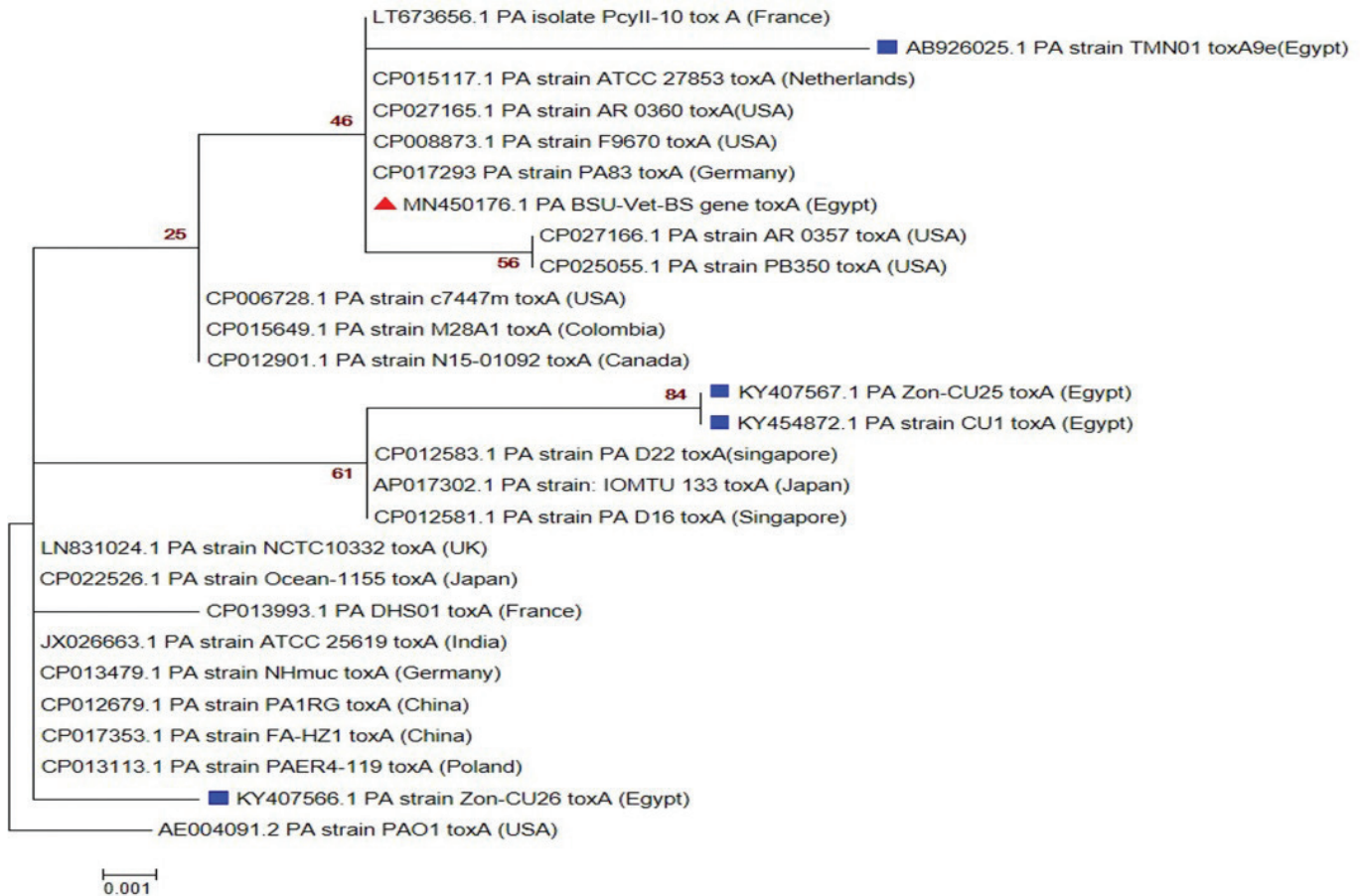
Strains	Nucleotides											
	No	1	2	3	4	5	6	7	8	9	10	11
AE004091.2 (USA)	1		99.58	99.24	98.02	99.07	98.02	95.42	98.75	99.22	98.96	99.16
LN831024.1 (UK)	2	99.84		99.50	98.31	99.38	98.31	95.69	98.64	99.53	98.85	99.27
MN450176.1 (This study)	3	100.0	100.0		98.58	99.06	98.58	96.23	100.0	99.50	99.5	99.75
KY407567.1 (Egypt)	4	99.15	99.15	99.15		98.44	100.0	97.46	98.31	98.31	98.31	98.59
KY407566.1 (Egypt)	5	99.06	99.06	99.06	99.06		98.44	97.82	98.75	99.38	99.38	99.07
KY454872.1 (Egypt)	6	99.15	99.15	99.15	100.0	99.06		97.46	98.31	98.31	98.31	98.59
AB926025.1 (Egypt)	7	80.00	80.00	80.00	82.91	90.57	82.91		96.23	95.69	95.69	95.96
CP017293.1 (Germany)	8	98.43	98.59	100.0	99.15	99.06	99.15	80.00		98.28	99.06	98.54
CP013113.1 (Poland)	9	99.53	99.69	100.0	99.15	99.06	99.15	80.00	98.28		98.59	99.11
CP022526.1 (Japan)	10	99.06	99.22	100.0	99.15	99.06	99.15	80.00	99.06	98.90		98.64
CP012901.1 (Canada)	11	99.22	99.37	100.0	99.15	99.06	99.15	80.00	98.28	99.37	98.90	
Aminoacids												

**Table 4.** Nucleotides and amino acids identity of *mexR* gene sequences of *P. aeruginosa*.

Strains	Nucleotides											
	No	1	2	3	4	5	6	7	8	9	10	11
AE004091.2 (USA)	1		100.0	100.0	82.23	83.22	83.11	99.77	99.55	99.77	99.77	99.55
LT969520.1 (UK)	2	100.0		100.0	82.23	83.22	83.11	99.77	99.55	99.77	99.77	99.55
MN450177.1 (This study)	3	100.0	100.0		82.23	83.22	83.11	99.77	99.55	99.77	99.77	99.55
KU597741.1 (Egypt)	4	99.20	99.20	99.20		99.47	99.47	81.96	82.49	81.96	81.96	82.23
KU597742.1 (Egypt)	5	100.0	100.0	100.0	99.20		100.0	82.99	82.77	82.99	82.99	82.77
KU597743.1 (Egypt)	6	100.0	100.0	100.0	99.20	100.0		82.88	82.66	82.88	82.88	82.66
CP013479.1 (Germany)	7	100.0	100.0	100.0	99.20	100.0	100.0		99.32	100	99.55	99.32
AY899300.1 (China)	8	99.32	99.32	99.32	100.0	99.32	99.32	99.32		99.32	99.32	99.55
CP012901.1 (Canada)	9	100.0	100.0	100.0	99.20	100.0	100.0	100.0	99.32		99.55	99.32
CP015001.1 (Brazil)	10	99.32	99.32	99.32	98.40	99.32	99.32	99.32	98.64	99.32		99.32
CP015649.1 (Colombia)	11	100.0	100.0	100.0	99.20	100.0	100.0	100.0	99.32	100.0	99.32	
Aminoacids												

**Table 5.** Nucleotides and amino acids identity of *lasI* gene sequences of *P. aeruginosa*.

Strains	Nucleotides											
	No	1	2	3	4	5	6	7	8	9	10	11
AE004091.2 (USA)	1		100.0	100.0	99.47	99.30	99.30	99.65	99.65	99.48	100.0	100.0
LT969520.1 (UK)	2	100.0		100.0	99.47	98.94	96.82	94.70	93.11	92.58	91.52	90.99
MN450178.1 (This study)	3	100.0	100.0		99.43	99.25	99.25	99.62	99.62	99.44	100.0	100.0
KP998820.1 (Egypt)	4	100.0	100.0	100.0		100.0	99.64	99.64	99.47	99.47	99.47	99.47
KP998821.1 (Egypt)	5	99.47	99.47	99.44	100.0		99.3	99.3	99.47	99.48	99.30	99.30
KR020718.1 (Egypt)	6	98.95	98.95	98.88	100.0	99.47		99.65	99.82	99.48	99.30	99.30
KR020722.1 (Egypt)	7	99.47	99.47	99.44	100.0	99.47	99.47		99.82	99.47	99.65	99.65
KR020725.1 (Egypt)	8	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	99.65	99.65
KR020726.1 (Egypt)	9	99.48	99.48	99.44	100.0	100.0	99.47	99.47	100.0		99.48	99.48
CP015647.1 (Colombia)	10	100.0	100.0	100.0	100.0	99.47	98.95	99.47	100.0	99.48		100.0
CP016955.1 (Netherlands)	11	100.0	100.0	100.0	100.0	99.47	98.95	99.47	100.0	99.48	100.0	
Aminoacids												



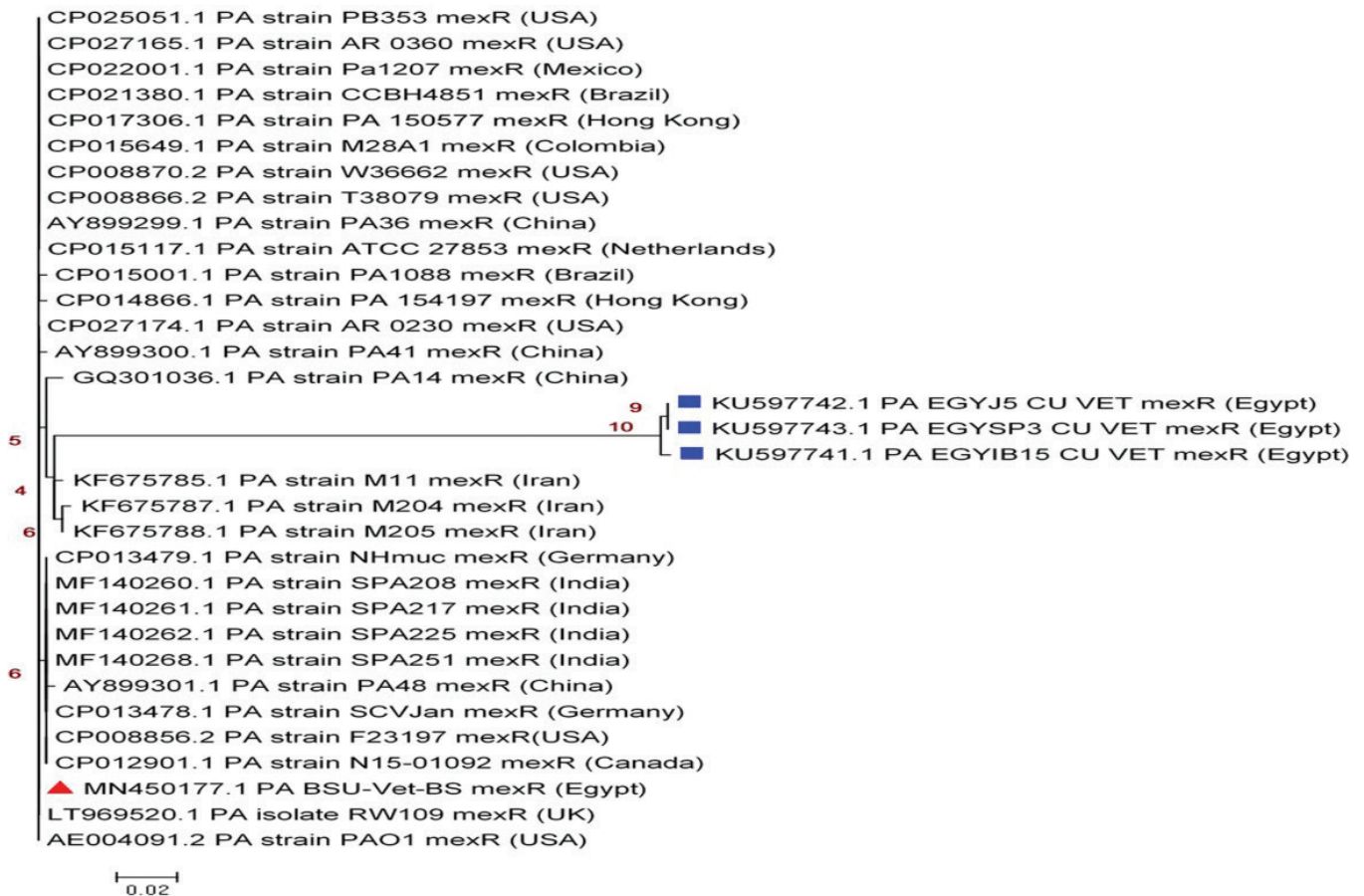
**Figure 8.** Phylogenetic analysis of *P. aeruginosa* *toxA* gene. Maximum likelihood tree of 1,000 bootstrap value was conducted after the Clustal W alignment algorithm using MEGA X program. Strain presented in this study is labeled with red triangle while other Egyptian strains are marked with blue square.

isolates to amikacin could be supported by a former study used amikacin as monotherapy for sepsis caused by pan-resistant *P. aeruginosa* [23]. *P. aeruginosa* strains showed a high degree of sensitivity to polymyxin-B, and this result coincides the previous record [24]. A previous study [25] recommended cefepime for the treatment of *P. aeruginosa* infections that agree with the current results. On the other hand, all the tested isolates are entirely resistant to various antimicrobial agents (ampicillin, doxycycline, ceftazidime, florfenicol, lincomycin, and sulfamethoxazole/trimethoprim), followed by erythromycin (95.5%) and cefotaxime (91.2%). All *P. aeruginosa* isolates under test showed an MDR pattern as they resisted at least a single agent from four classes and extended to 14 of the tested antimicrobial classes within 5 (11.11%) of the isolates under test. Multidrug-resistant bacteria are deliberated now as an authentic hazard in human and/or veterinary medicine [26]. *P. aeruginosa* is naturally resistant to most antimicrobials and has numerous intrinsic mechanisms to decrease

its susceptibility to them as well; it commonly attains added resistance mechanisms and usually develops multidrug resistance during a treatment regimen [27].

The genotypic investigation of antimicrobial resistance and virulence genes of *P. aeruginosa* of poultry origin is scanty, so one of these studies aims was to detect the occurrence of antimicrobial resistance genes such as *bla*<sub>CTX</sub>, *fox*, and *mexR* genes and virulence genes such as *toxA*, *exoY*, *lasB*, and *lasI* genes among *P. aeruginosa* isolates. Concerning resistance genes, *bla*<sub>CTX</sub> was detected in 100% of the examined *P. aeruginosa* isolates although the lower prevalence was recorded by others [28,29]. The detection of *bla*<sub>CTX</sub> gene among all the tested isolates could explain the growing resistance pattern of *P. aeruginosa*, which reached to complete resistance against the third generation of cephalosporins represented by cefotaxime. In relation to *fox* gene, it was noticed in 80% of the isolates, but this result was higher than previously reported by Voolaid et al. [30], who detected the *fox* gene in 25% of the tested *P.*



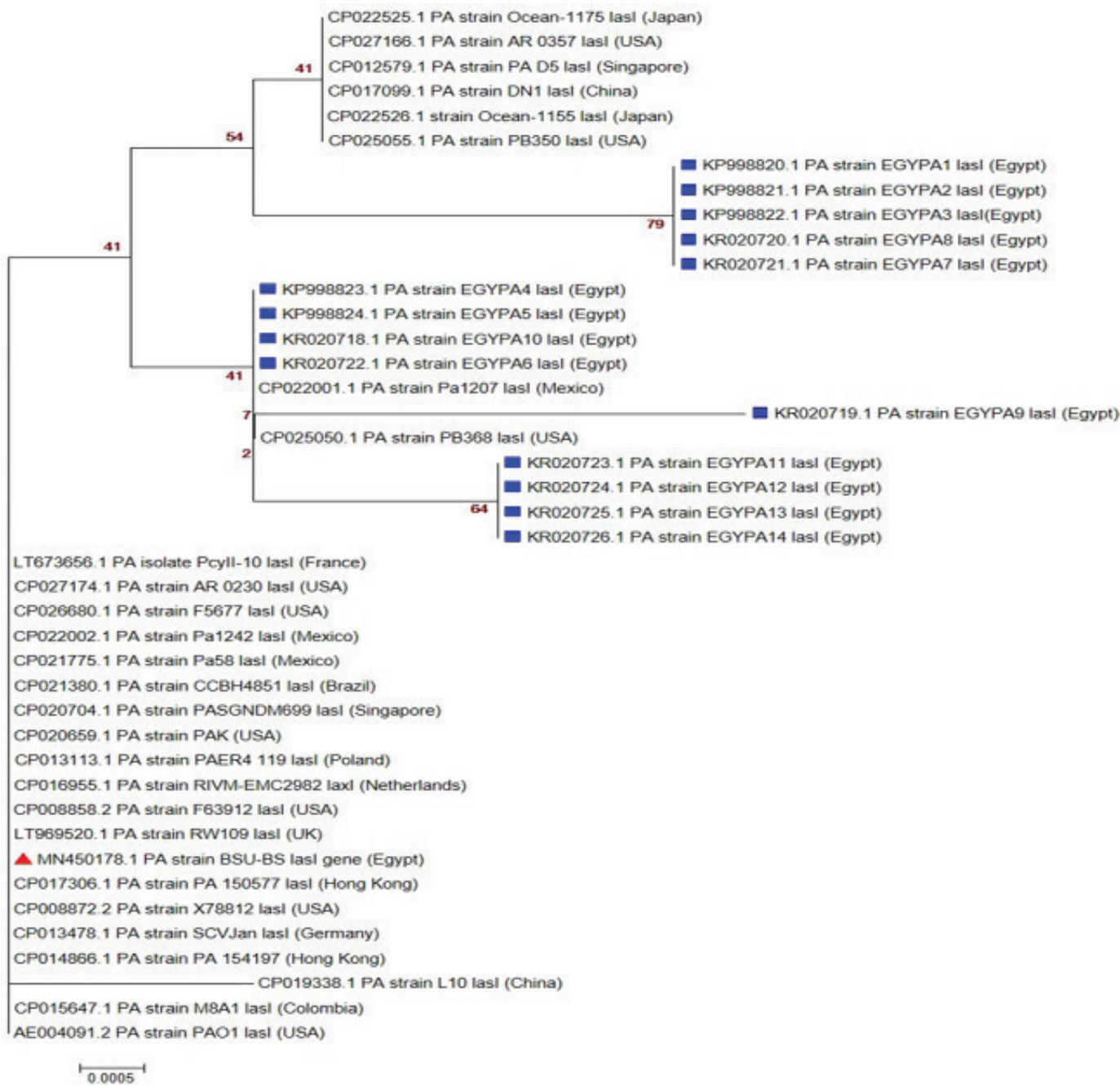


**Figure 9.** Phylogenetic analysis of *P. aeruginosa mexR* gene. The maximum likelihood tree of 1,000 bootstrap value was conducted after the Clustal W alignment algorithm using MEGA X program. Strain presented in this study is labeled with a red triangle, while other Egyptian strains are labeled with blue square.

*aeruginosa* isolates. This high prevalence of *fox* gene in this work may elucidate the rising resistance behavior, which reached to complete resistance against the second-generation cephalosporins represented by cefoxitin. Regarding *mexR* gene, it was identified in 100% of the isolates, and this may explain why all the recovered *P. aeruginosa* isolates in the present work showed a multidrug-resistant pattern, which represents a high risk in the treatment of pseudomonal infection not only in poultry diseases but also with human infection. Quorum sensing is a bacterial way of communication through specific chemical molecules to regulate their action against hosts through the production of virulence factors and antimicrobial-resistant determinants [31]. One of the most critical virulence genes is *toxA*, which found in 100% of the studied isolates, and this result coincides with several studies [24,32,33]. *ToxA* gene can be used as a marker to identify *P. aeruginosa* by using PCR [32]. The existence of *toxA* gene among *P. aeruginosa* clinical isolates follows the essential role of this virulence factor in

chicken respiratory affections [34]. *ExoY* gene was detected in 60% of the investigated isolates; a higher prevalence was recorded [9] with exoenzyme-Y, which is being implicated in the pathogenesis of *P. aeruginosa*. Among the tested isolates, *lasB* gene was detected in 80%, and this prevalence is nearly close to that previously described by Benie et al. [35]. Elastase (*LasB*) is an extracellularly secreted endopeptidase which regarded as an important virulence factor of *P. aeruginosa* [36]. Finally, *lasI* gene (autoinducer) was perceived in 80% of the isolates, which is responsible for the secretion of some virulence tools of *P. aeruginosa* as elastase and pyocyanin [8].

Amino acid and nucleotide sequence analysis revealed a high correlation most of the time with other *P. aeruginosa* sequences published on the GeneBank representing different clinical lesions, hosts, and localities, worldwide, especially with *P. aeruginosa* reference strain (PAO1). These findings were explained by Klockgether et al. [37]. They clarified that the core genome of *P. aeruginosa* is



**Figure 10.** Phylogenetic analysis of *P. aeruginosa lasI* gene. The maximum likelihood tree of 1,000 bootstrap value was conducted after the Clustal W alignment algorithm using the MEGA X program. Strain presented in this study is labeled with a red triangle while other Egyptian strains are labeled with blue square.

exceptionally conserved, and the relationships of strains from the same clonal complex, nevertheless discrete geographic origin, illustrate a low substitution rate.

*P. aeruginosa* can induce variable clinical lesions in different localization sites due to its inhibitory effect on the

cytokines production that reduces the host's ability to clear infection [38]. Therefore, the probability of transferring the highly virulent strains of this microorganism from broilers to human with zoonotic losses represents public health hazard.

## Conclusion

*Pseudomonas aeruginosa* that was isolated from diseased broilers makes available proof that poultry environment could constitute a public health risk due to the existence of multiple antibiotic resistances by several strains in this study, in addition to the distribution of both virulence and resistance genes among them.

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Nothing to disclose.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' contribution

First and the last author designed the work, second and last author carried the practical part, the third author carried out the sequencing part, the last author has written the manuscript, and other authors reviewed the writing.

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