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BIOCHEMICAL STUDIES ON ANTIBIOTIC RESISTANT AND VIRULENCE GENES OF *PSEUDOMONAS AERUGINOSA*, ISOLATED FROM MASTITIC COWS' MILK AND ITS ALTERATION ON BLOOD BIOCHEMICAL PARAMETERS

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ABSTRACT

The emerged antibiotic resistant bacteria issue is considered as an important problem that threats human's life including; health and agriculture affairs. *Pseudomonas aeruginosa* is one of the bacteria that affecting milk production and responsible for mastitis in cattle. Out of 50 mastitic milk samples, only 6 samples were found to be positive for *Pseudomonas aeroginousa* with an incidence of 12%. The PCR detection of *Pseudiomonas aeroginosa* antibiotic resistance gene *blaVIM* and the virulence gene *toxA*, indicated that only 4 isolates showed to be positive for *blaVIM* gene. While, all the 6 positive milk *Pseudomonas aeroginousa* isolates showed to be positive for *toxA* gene. The antibiotic sensitivity results showed that the isolates were resistant to Tetracycline, Piperacillin and Oxacillin and Piperacillin. However, these isolates were sensitive to Amikacin, Ceftazidime Cefoperazone and Impenem. Also, the infected animal serum biochemical parameters when compared to the healthy uninfected animals (control group), showed an elevation in the values of total protein, globulin, triglyceride, alanine aminotransferase, aspartate aminotransferase, uric acid and creatinine**.** While it showed decline in the values of albumin, glucose, cholesterol, low density lipoprotein, and high density lipoprotein.

Key words: Antibiotic resistance genes, Blood parameters, PCR, *Pseudomonas aeruginosa*, Mastitic cows and Virulence genes.

INTRODUCTION

In the last few decades it was obviously seen that antibiotics were not used for the treatments of human and animals only but it was used for non-therapeutic purposes. Since, these antibiotics were being used as growth promotors and prophylactic agents in dairy herds **(Aarestrup *et al.*, 2001; Marshall and Levy, 2011; World Health Organization (WHO) 2014; Food and Agriculture Organization (FAO), 2015 and Sharma *et al.*, 2017)**. As a result of the massive and intensive use of antibiotics in many fields of human's life including; health and agriculture such as poultry and animal farms, it emerged as an important issue for the entire world (**Hirsch and Tam, 2010, Sharma *et al.*, 2017 and Shrivastava *et al.*, 2018)**. WHO, reported that bacteria acquired resistance against antibiotic, which became "a global threaten" **(Potron *et al.*, 2015; Laxminarayan *et al.*, 2016 and Sharma *et al.*, 2017).** Even starting from 2014 the WHO reported that the world is running out of antibiotic because bacteria are continuously modifying themselves according to the new generations of antibiotics **(WHO, 2014). McManus (1997) and Wilson (2014),** indicated that bacteria have the ability to develop their resistance against antibiotics by at least one of the following mechanisms; altering the antibiotic target site, inactivating or modifying the antibiotic itself, promoting the efflux of antibiotic and adjusting the bacterial metabolic pathway so they could overcome the antibiotic effects. **McAdam *et al.*, (2012) and WHO, (2014)** illustrated the impacts of the bacteria which have one or multi-drug resistance (MDR) against antibiotics. This resistance may results in less effective antibiotics, long treatment duration, more costs of treatments and more needs for new agents which are more toxic and expensive. Also, the multi-drug resistance against antibiotics could be acquired by mutation of the bacterial existing genes or by horizontal gene transfer **(Summer 2006; Palmer *et al.*, 2010 and Gomez *et al.*, 2017)**. The main problem is the ability of these MDR bacteria to be transferred from food especially, animal-derived products and milk to human **(Angulo *et al.*, 2004, McAdam *et al.*, 2012 and Sharma *et al.*, 2017).** And milk is considered as an important natural high nutritional and popular healthy food for all ages all over the world **(Krehbiel, 2013; Handford *et al.*, 2016 and**

O'Callaghan *et al.*, 2019). Thus, the production of this valuable food is facing many problems which are affecting the quality and the quantity of milk production around the world causing economics' losses such as mastitis. From the bacteria that affecting milk production and responsible for mastitis in cattle is *Pseudomonas aeruginosa* **(Dinaol *et al.*, 2016; Ghassan and Hassan, 2016; Anjali and Kashyap, 2017, and Abdalhamed *et al.*, 2018)**. *Pseudomonas aeruginosa* is an opportunistic pathogen that causing diseases for both human and animals **(Xavier *et al.*, 2010).** This pathogen could survive under hard conditions of nutrition and moisture, causing sudden outbreak within short period in dairy farms and it remains in the udder of the animal for years **(Bengtsson *et al.*, 2009; Park *et al.*, 2014; Razzaq *et al.*, 2018; Anjali and Kashyap, 2017, and Abdalhamed *et al.*, 2018)**. It was found that *P. aeruginosa* continuous is a resistant micro-organism because of its high intrinsic resistance to most classes of antibiotics **(Seol *et al.*, 2002 and Wolf and Elsasser, 2009).** Also, there are many genes which are responsible for these bacterial antibiotics' resistance such as *mexR* and *blaVIM*, and virulence such as *exoS* and *toxA* that were detected in many *P. aeruginosa* isolates (**Khattab *et al.*, 2015; Ghassan and Hassan, 2016; Ibrahim *et al.*, 2017; and Sanz-Garcia *et al.*, 2018).** The multi-drug resistance of *Pseudomonas aeruginosa* is depending on the expression of the metallo-β-lactamases, which is encoded by the *blaVIM* gene **(Sader *et al.*, 2005; Tato *et al.*, 2010; Khorvash *et al.*, 2017 and Mombini *et al.*, 2019).** The way these enzymes inactivate the β-lactam classes of antibiotics is by removing the zinc ion from the active site of the enzyme or catalyzing the β-lactam ring hydrolysis of antibiotics **(Rotondo and Wright, 2017, and Palacios *et al.*, 2018).** Also, *Pseudomonas aeruginosa* bacteria excrete exotoxins such as exotoxins A which acts as a major systemic virulence factor for these bacteria which causes specific protein biosynthesis and cell death **(Amira, 2018; Cavaillon, 2018 and Moss *et al.*, 2019).** And exotoxin A is responsible for local tissue damage, bacterial invasion, and immunosuppression **(Ertugrul *et al.*, 2017 and Frantisek *et al.*, 2017).** Besides, the biochemical and immunological parameters of the bacterial hosts were affected by these genes **(Ibrahim *et al.*, 2017 and Moss *et al.*, 2019).** So, the main aim of present work is to biochemically characterize the antibiotic resistance and virulence genes of *blaVIM* and *toxA* produced by *Pseudomonas aeruginosa* after its isolation from clinically mastitic milk. And, identify its antibiotic sensitivity using Vitek2 compact system. Besides, studying the alteration of these bacteria on hosts' blood parameters.

MATERIALS AND METHODS

Samples

Samples were collected from apparently mastitic cows raised in private farms in Fayoum Governorate, Egypt. Both milk and blood samples were collected from each animal in the morning during the period started from April: June 2018.

Milk samples

A total of 50 milk samples were collected from apparently mastitic cows. These samples were collected under complete hygienic measure in sterilized bottles and transferred to the laboratory in ice box according to (**Quinn *et al.*, 2002**).

Serum biochemical parameters analyses:

Blood samples were collected from the jugular vein from each animal in 3 replicates. Six ml of blood each sample was transferred to non-heparinized tubes and was centrifuged at 3000 rpm for 15 min at 4 ˚C. Serum samples were collected, and kept directly in ice then, kept in the deep freezer at -20 ˚C till the next analyses. All the selected biochemical parameters analyses were done using commercial ready-kits from Biodiagnostic Inc, Dokki, Giza, Egypt. The determination of total protein (TP) and albumin (ALB), were done according to the methods described by **(Doumas *et al.*, 1981)** and **Drupt (1974),** respectively. And, the calculation of the difference between the total protein and albumin concentrations gave the globulin concentration according to **Coles (1986).** Glucose (GLU), was determined as described by **Trinder (1969).** The method of **Reitman and Frankel, (1957)** was used for the determination of both **A**spartate aminotransferase (AST) and Alanine aminotransferase (ALT)**.** Triglycerides (TG), cholesterol (CHOL), high density lipoprotein (HDL), were determined as described by **(Zollner and Kirsch, 1962). Friedewald *et al.*, (1972)** method was used to estimate the Low density lipoprotein (LDL). Uric acid was measured as described by **(Barham and Trinder, 1972)**.Creatinine was measured according to (**Bartles *et al.*, 1972).** All the calculation, standard curves and assays were done according to the kits' protocols.

***Pseudomonas aeroginousa* isolation and identification from milk samples:**

Samples

Milk samples were centrifuged at 3000 rpm, and then incubated aerobically for 24 hours (h) at 37 °C. A loopful from each sample was

cultured onto blood agar and MacConkey agar plates. Then, they incubated aerobically at 37 °C for 24 h. The growing *Pseudomonas aeroginousa* colonies were identified morphologically and biochemically according to **(Quinn and Markey, 2003).**

Biochemical identification of *Pseudomonas aeroginousa* using Vitek2 compact system:

Pseudomonas aeroginousa biochemical identification was done using Vitek2 compact system 08.01 (bioMérieux, Durham, NC, USA), according to the manufacturer's instructions, using the Gram-Negative (GN) card which is a complete system for routine identification testing of most clinically significant Gram-Negative organisms **(Chatzigeorgiou *et al.*, 2011).** A 0.45 % saline solution (pH 4.5:7.0) was used with the morphologically similar colonies to prepare the organism suspension. The last suspension was calibrated using VITEK® 2 DensiCHEK™ Plus to a final density equivalent to a 0.50 to 0.63 McFarland. Then, the last organism suspension was used to fill the test cards for Vitek2 instrument.

***In vitro* antibiotic sensitivity test of *Pseudomonas aeroginousa* by Vitek2:**

In vitro antibiotic sensitivity test of *P. aeroginousa* identification was done using Vitek2 compact system and antimicrobial susceptibility Gram-negative test card (AST-GN card) according to the manufactures' instructions **(Chatzigeorgiou *et al.*, 2011).** For each tested isolate, the identification AST-GN cards were inoculated with the microorganism suspension. The card identified different 47 biochemical tests. A test tube containing the microorganism suspension was placed into a special rack (cassette). The filled cassette was placed into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells. The antibiotic sensitivity test of *Pseudomonas aeroginousa* was done against Amikacin, Cefotaxime, Ciprofloxacin, Ceftriaxone, Cefoperazone, Ceftazidime, Gentamicin, Impenem, Oxacillin, Piperacillin, and Tetracycline.

PCR detection of *blaVIM* and *toxA* genes in *Pseudomonas aeroginousa* isolates:

DNA extraction from four random +ve *Pseudomonas aeroginousa* isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the instructions of the manufacturer and **Sambrook *et al.*, (1989).** Briefly, 200 µl of the sample suspension was added to 10 µl of the proteinase K and 200 µl of the lysis buffer and

incubated at 56˚C for 10 min. Then, 200 µl of 100% ethyl alcohol was added to the lysate. After washing and centrifuging the sample, 100 µl of elution buffer that provided by the kit was used to elute the nucleic acid.

PCR amplification:

Primers were used for the PCR amplification for *blaVIM and toxA* genes. These primers were utilized for the PCR reaction and for the analysis using forward and reverse PCR primers for *blaVIM* gene which was done according to **Noori *et al.*, (2013)** as shown in **Table (1).** And for *toxA* gene it was done according to **(Khan and Cerniglia, 1994**) as shown in **Table (1).** These primers were utilized in a 25 µl reaction containing 12.5 µl of PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of nuclease-free water, and 6 µl of DNA template. The reaction was performed in an (Applied Biosystem Thermal Cycler). Cycling conditions of the different primers during the PCR amplification as the manufacturer's recommendations as follow: primary denaturation: 94˚C-5 min., secondary denaturation: 94˚C-30 sec., annealing: 55˚C-45 sec., extension: 72˚C-45 sec., no. of cycles: 35 and final extension: 72˚C-10 min.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. A gelpilot 100bp and 100bp plus DNA Ladders (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Table (1): Target genes, primers sequences, and amplicons sizes.

Target gene	Primers sequences Forward (F) Reverse (R)	Amplified segment (bp)
blaVIM	F:TTTGGTCGCATATCGCAACG	500
	R: GGCATTCGCCAGTAAGCGG	
toxA	F:GACAACGCCCTCAGCATCACCAGC	396
	R:CGCTGGCCCATTCGCTCCAGCGCT	

Statistical analysis:

The results were subjected to statistical analysis using One-way ANOVA, for the determination of the significant differences between the

groups' mean according to **(Petrie and Watson, 2013).** The data are showed as means ± standard error of the mean. Probability values less $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Prevalence of *Pseudomonas aeroginousa* isolated from milk samples:

Six isolates of *P. aeruginosa* were isolated from fifty clinically mastitic cow's milk samples in an incidence of 12% as shown in **Table (2).**

Table (2): Prevalence of *Pseudomonas aeroginousa* isolated from clinically mastitic milk

Total examined mastitic milk samples	Pseudomonas isolates	
	No. of +ve isolates	% +ve isolates
50	6	12

No.: Number of the positive (+ve) examined mastitic milk samples and %: Percentage in relation to No. of total examined mastitic milk samples (50).

Biochemical identification of *Pseudomonas aeroginousa* isolates by Vitek2 system is shown in **Table (3).** This identification of *Pseudomonas aeroginousa* was done using Vitek2 compact system 08.01 with identification confidence and probability of 98.7%.

Table (3): Biochemical details of *Pseudomonas aeroginousa* using Vitek 2 compact system

Table 3: Biochemical details of *Pseudomonas aeruginosa*																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTP	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	+
23	proA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTER	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATK	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

proA: L-ProlineArylamidase, ILATK: L-Lactate alkalinization, LIP: Lipase, SUCT: Succinate alkalinization, dGLU: D-glucose, TyrA: Tyrosine Arylamidase, CIT: Sodium Citrate, GGT: γ-Gutamyl- Transferase, MNT: Malonate, CMT: Coumerate and BAlap: β-Alanine arylamidasepNA. The identification was done using vitek2 compact system 08.01 with probability of 98.7%.

The results of antibiotic sensitivity test:

The results showed that the isolates were resistant to Tetracycline, Piperacillin and Oxacillin and Piperacillin. While these isolates were sensitive to Amikacin, Ceftazidime Cefoperazone and Impenem as shown in **Table (4).**

Table (4): Antibiogram pattern of *P. aeruginosa* isolates against different antibiotics

Antibiotic	Strength potency	Sensitive		Intermediate		Resistant	
		No	%*	No	%*	No	%*
Amikacin	30 µg	5	83.33	0	0.00	1	16.66
Ceftazidime	30µg	5	83.33	0	0.00	1	16.66
Cefotaxime	30µg	2	33.33	0	0.00	4	66.66
Cefoperazone	50µg	5	83.33	0	0.00	1	16.66
Ceftriaxone	30µg	3	50.00	1	16.66	2	33.33
Ciprofloxacin	5µg	4	66.66	0	0.00	2	33.33
Gentamicin	120µg	4	66.66	0	0.00	2	33.33
Impenem	10µg	5	83.33	0	0.00	1	16.66
Oxacillin	1µg	1	16.66	0	0.00	4	83.33
Piperacillin	100µg	1	16.66	0	0.00	4	83.33
Tetracycline	30µg	1	16.66	0	0.00	5	83.33

*Percentages were calculated according to the No. of tested isolates (6).

Detection of *Pseudomonas aeruginosa* antibiotic resistant and virulence genes using PCR:

The detection of *Pseudiomonas aeroginosa* antibiotic resistance gene based on *blaVIM* primers giving amplicon at 500bp for *blaVIM* gene as in **Fig (1).** Out of the 6 positive milk *Pseudomonas aeroginousa* isolates, only 4 isolates showed to be positive for the *blaVIM* antibiotic resistant gene. And the detection of *Pseudiomonas aeroginosa toxA* virulence gene based on *toxA* primers which gives amplicon at 396bp for *toxA* gene is shown in **Fig (2).** All the 6 positive milk *Pseudomonas aeroginousa* isolates showed to be positive for the *toxA* gene virulence gene.

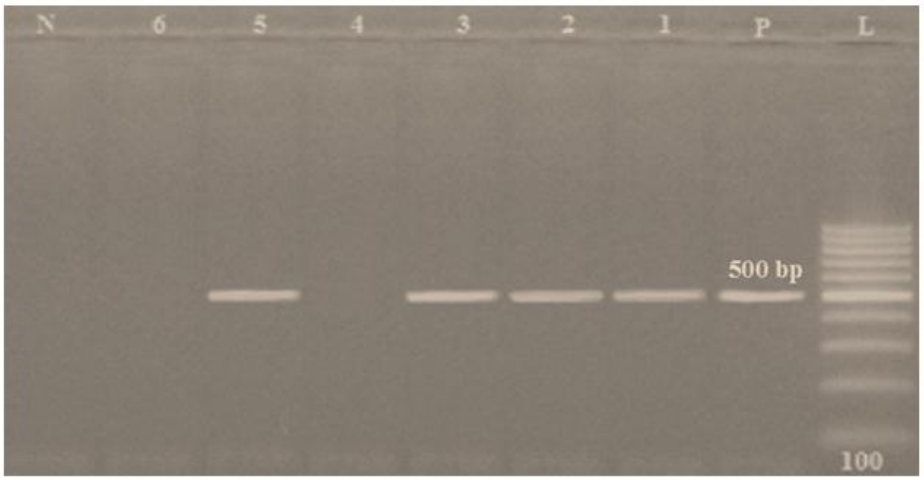

Fig (1): Agarose gel electrophoresis of multiplex PCR amplification of *Pseudomonas aeroginousa* extracted DNA for *blaVIM* gene, L: represents the molecular size marker (DNA ladder), N.: Negative control, P.: Positive control of *blaVIM* (500bp), Lanes: 1, 2, 3 and 5 are positives for *blaVIM* gene. And, lanes 4 and 6 are negatives for *blaVIM* gene.

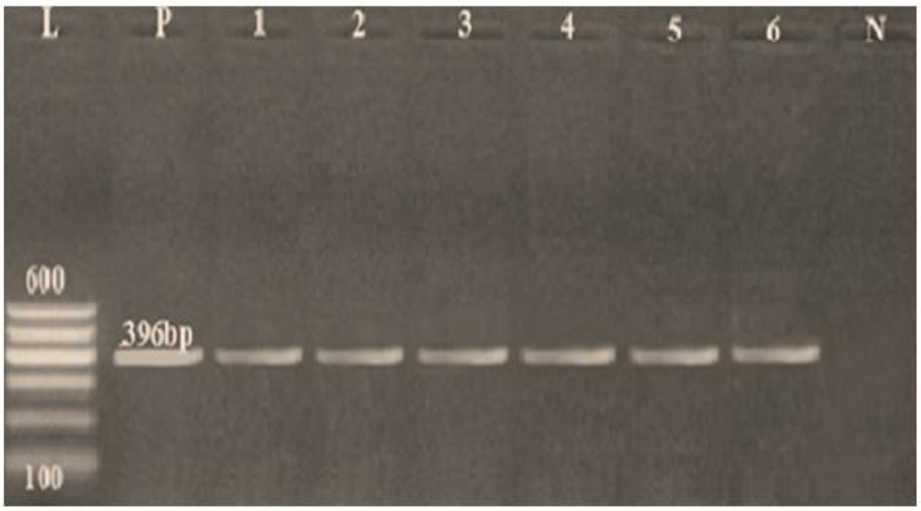

Fig (2): Agarose gel electrophoresis of multiplex PCR amplification of *Pseudomonas aeroginousa* extracted DNA for *toxA* gene, L: represents the molecular size marker (DNA ladder), N.: Negative control, P.: Positive control of *toxA* (396bp) and Lanes: 1, 2, 3, 4, 5 and 6 are positives for *toxA* gene.

Serum biochemical analyses:

Out of the fifty apparently mastitic cows, only six milk samples showed to be infected with *Pseudomonas aeruginosa*. So, the animals of the infected milk samples were subject for further blood biochemical analyses. Results in **Table (5)** showed that the biochemical parameters were altered as a cause of *Pseudomonas aeruginosa* infection. The infected animal serum biochemical parameters when compared to the healthy uninfected animals (control group), showed elevation in the values of total protein, globulin, triglyceride, alanine aminotransferase, aspartate aminotransferase, uric acid and creatinine**.** These values were 6.43±0.04 g/dl, 1.30±7.0 g/dl, 80.10±0.31 mg/dl, 22.53± 0.27 U/L, 61.31± 0.65 U/L, 24.25± 0.96 mg/dl and 0.91± 0.04 mg/dl, respectively. While the infected animal serum biochemical parameters when compared to the healthy uninfected animals (control group), showed decline in the values of albumin, glucose, cholesterol, low density lipoprotein (LDL), and high density lipoprotein (HDL). These values were 1.30±7.0 g/dl, 68.19±0.98 mg/dl, 175.95±1.87 mg/dl, 52.27±0.64 mg/dl and107.66±0.78 mg/dl respectively. All the pervious values were significant ($p < 0.05$), except for the glucose value was not significant ($p > 0.05$).

Table (5). Serum biochemical indices in cows infected with *Pseudomonas Aeruginosa* (Mean ± SEM)

Serum biochemical parameters		
Serum parameters	**Control**	**Infected cows with**
Total protein g/dL	5.98 ±0.09[a]	6.43±0.04[b]
Albumin g/dL	1.62 ±8.0[a]	1.30±7.0[b]
Globulin g/dL	4.37 ±0.07[a]	5.13±0.10[b]
Glucose mg/dL	74.58±1.01[a]	68.19±0.98
Cholesterol mg/dL	179.01 ±1.21[a]	175.95±1.87[a]
Triglyceride mg/dL	70.75 ±0.26[a]	80.10±0.31[b]
Low density lipoprotein (LDL) mg/dL	55.70 ± 0.87[a]	52.27±0.64[b]
High density lipoprotein (HDL) mg/dL	109.16 ± 0.91[a]	107.66±0.78[b]
Alanine aminotransferase (ALT) U/L	19.02 ± 0.30[a]	22.53± 0.27[b]
Aspartate aminotransferase (AST) U/L	59.37 ± 0.24[a]	61.31± 0.65[b]
Uric acid mg/dl	22.73± 0.34[a]	24.25± 0.96[b]
Creatinine mg/dl	0.83± 0.02[a]	0.91± 0.04[b]

Means followed by different letters in the same row differ significantly ($p < 0.05$).
SEM: Standard Error Means.

The present work is focusing on using the multiplex PCR to detect the incidence antibiotic resistance and virulence genes of *blaVIM* and *toxA* which produced by *Pseudomonas aeruginosa* after its isolation from clinically mastitic milk. Out of the total 50 mistatic milk samples only 6 samples were found to be positive for *Pseudomonas aeroginousa* with an incidence of 12%. This percentage is lower than the results obtained by **(Gangwal and Kashyap, 2017 and Ibrahim *et al.*, 2017**) who isolated *Pseudomonas aeroginousa* with an incidence of 18.8% and 34%, respectively. The prevalence rate may vary from place to another according to the hygienic practices, geographic area and bacterial strains **(WHO, 2014 and Faith *et al.*, 2015).** These isolates were identified

morphologically, biochemically using regular tests and further biochemical identification of *Pseudomonas aeroginousa* was done using vitek2 compact system 08.01. **(Funke and Funke-Kissling, 2004 and Hackman *et al.*, 2013),** indicated that Vitek2 system is reliable and accurate for the detection of Gram-negative bacteria including *Pseudomonas aeroginousa***.**

The results showed that the isolates were resistant to Tetracycline, Piperacillin and Oxacillin and Piperacillin. While these isolates were sensitive to Amikacin, Ceftazidime Cefoperazone and Impenem as shown in **Table (4)** which is in agreement with (**El-Sebaey *et al.*, 2018)**.The detection of *Pseudiomonas aeroginosa* antibiotic resistance gene *blaVIM* showed that out of the 6 positive milk *Pseudomonas aeroginousa* isolates, only 4 isolates showed to be positive for *blaVIM* gene as in **Fig (1).** This result is in accordance with (**Ibrahim *et al.*, 2017; Khorvash *et al.*, 2017; Chairat *et al.*, 2019 and Mombini *et al.*, 2019**) who found that 75% of the *Pseudiomonas aeroginosa* isolates were positives for the previous gene. And the detection of *Pseudiomonas aeroginosa toxA* virulence gene showed that all the 6 positive milk *Pseudomonas aeroginousa* isolates showed to be positive for this gene which in line with **(Xu *et al.*, 2004; Khattab *et al.*, 2015; Amira, 2018 and Aljarah, 2018).** The important issue is the ability of bacteria to develop their resistance against antibiotics by many mechanisms which enable it to overcome the antibiotic effects **(McManus, 1997 and Wilson, 2014).** Besides, the emerged virulence that acquired by the virulence genes of bacteria is responsible for local tissue damage, bacterial invasion, immunosuppression and altering the biochemical parameters of the bacterial hosts, resulting in more virulence and antibiotic resistance of the bacteria **(Ertugrul *et al.*, 2017; Frantisek *et al.*, 2017; Ibrahim *et al.*, 2017 and Moss *et al.*, 2019).** So, the animal blood biochemical parameters are affected by the infection of bacteria as well. And, serum biochemical parameters used to give an indicator of both animal and human health status **(Radkowska *et al.*, 2014; Wenping *et al.*, 2015 and Salisu *et al.*, 2018).** In present study, the total protein result was higher in the infected animals with *Pseudomonas aeroginousa* than the healthy animal (control group) as shown in **Table (5).** The higher total protein may be due the elevated catabolism of it and its absorption is reduced **(Mercier *et al.*, 2002; Pandey *et al.*, 2011; Alberghina *et al.*, 2015 and Sarvesha *et al.*, 2016).** Also, it is noticeable that globulin was higher in the infected animal comparing with the control group. This may be a result of the inflammatory status caused by the bacteria, causing to

elevate some protein such as globulin as a response and decreasing the albumin/globulin ratio which was 0.37 in the control group vs 0.25 in the infected group is in accordance with **(Pandey *et al.*, 2011; Singh *et al.*, 2014 and Ibrahim *et al.*, 2017).** Also, cholesterol was elevated and triglyceride was decreased in the infected animal which opposing with the control as shown in **Table (5)** which is in agreement with (**Ibrahim *et al.*, 2017)**. In infection and/or inflammatory response of animals, who provide the energy to the body via lipolysis, the may be the cause of low level of glucose in the infected group as well **(Feingold *et al.*, 1995, and Schreiber and Zechner, 2014).** So, the total protein and cholesterol together are decreased may due to inflammatory reaction and/or protein-losing enteropathy**. (Khovidhunkit *et al.*, 2000; 2004, and Nassaji and Ghorbani 2012),** indicated that cholesterol is a part of the lipoproteins such as HDL and LDL biosynthesis and the decrease in cholesterol biosynthesis means decrease of their levels together. Also, triglyceride in the infected animals was elevated may be due to it protective role in Gram-negative bacteria in the detoxification of lipopolysaccharide of the wall structure of the bacteria **(Feingold *et al.*, 1995 and Eckel and Ametaj, 2016).** Since, liver is the factory of lipids and lipoproteins syntheses, this may affect the level of both lipids profile indices and liver enzymes as well when liver is damage **(Sarvesha *et al.*, 2016).** The liver enzymes are indicators of liver function or damage which elevated as a cause of infection; this is in agreement with **(Pandey *et al.*, 2011 and Sarvesha *et al.*, 2016)**. The increased level of uric acid may be due the body protein catabolism resulting in too much production of urea in case of toxic conditions **(Mercier *et al.*, 2002 and Alberghina *et al.*, 2015).**

CONCLUSION

The emerged antibiotic resistant bacteria issue is considered as an important problem that threats human's life, including their health and agriculture (poultry and animals farms). *Pseudomonas aeruginosa* is one of the bacteria that affecting milk production and responsible for mastitis in cattle. The severity of mastitis in cows is greatly affected by the presence of antibiotic resistance and virulence genes in the causative *Pseudomonas aeruginosa* strains and this is easily detected by the application of PCR which is rapid and accurate technique for the detection of these genes mainly *blaVIM* and *toxA.* As it is previously mentioned that the infection with antibiotic resistant bacterial strains is affected and dramatically increases its severity when accompanied with virulence. So, hygienic measures should be applied in all humans' life and

especially in dairy farms. Also, miss use of antibiotics should be avoided, and their usage must be following accurate laboratory examination.

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دراسات كيميائيه حيويه علي جينات المقاومه للمضادات الحيويه و الضراوه من السيدومونس ارجينوزا المعزوله من لبن الابقار المصابه بإلتهاب الضرع و تأثيرها علي مكونات الدم

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تعتبر مشكلة البكتيريا المقاومة للمضادات الحيوية مشكلة هامه تهدد حياة الإنسان بما في ذلك ؛ الصحة والزراعة. و تعتبر السيدومونس ارجينوزا واحدة من البكتيريا التي تؤثر على إنتاج اللبن ومسئولة عن التهاب الضرع في الماشية. من بين 50 عينة من لبن ابقار المصابه بالتهاب الضرع ، تم عزل 6 عترات للسيدوموناس ارجينوزا بمعدل 12٪. بإستخدم تفاعل إنزيم البلمره المتسلسل للكشف عن وجود جين المقاومه للمضادات الحيويه *blaVIM* و جين الضراوه *toxA*، تبين إيجابيه 4 عترات فقط لجين *blaVIM* بينما كانت ال 6 عترات للسيدومونس ارجينوزا المعزوله من اللبن ايجابيه لجين الضراوه *toxA*. أظهرت نتائج أختبار حساسية المضادات الحيوية أن العزلات كانت مقاومة للتتراسكلين، البيبيراسيلين، أوكساسيلين والبيبيراسيلين. بينما كانت المعزولات حساسه لكلا من الاميكاسين، سيفتازيديم، سيفوبيرازون و امبنيم. أيضا، أظهرت التحاليل البيوكيميائية لمصل الحيوانات المصابة عند مقارنتها بالحيوانات غير المصابة (المجموعة الضابطة) ، ارتفاعًا في قيم البروتين الكلي، الجلوبيولين، الجليسريدات الثلاثي، ألانين أمينوترانسفيراز، الأسبارتات أمينوترانسفيراز، حمض اليوريك والكرياتينين. بينما أظهر انخفاضًا في قيم الألبيومين، الجلوكوز، الكوليسترول، الكوليسترول-منخفض الكثافة و الكوليسترول-عالي الكثافة