Animal Health Research Journal Vol. 7, No. 4, November 2019 pp. 503-513

ISSN: 2356-7767

Bovine mastitis associated with *Pseudomonas aeruginosa* and their molecular characterization

Abdelgawad, M.H. and Bkheet, A.A.

Animal Health Research Institute, Bacteriology Unit, Damanhour Branch
Animal Health Research Institute (AHRI), Agriculture Research Central (ARC)

Received in 04/10/2019
Accepted in 11/11/2019

Abstract

Bovine mastitis associated with *Pseudomonas aeruginosa* has been increased in recent years causing large economic losses and significant problems to dairy farms and small farmers as *P. aeruginosa* is naturally resistant to wide spectrum of antibiotics in addition to its possession to a variety of virulence factors that may related to its pathogenicity. This study was carried out on bovine mastitis accompanied with *P. aeruginosa* in which 170 milk samples from mastitic cows and buffalos (100 cow and 70 buffalo) were collected from different localities in El-Behera governorate. Molecular characterization (PCR) were developed to identify *P. aeruginosa*, detect some virulence genes that associated with pathogen city *(toxA , exoS and oprl genes)* and detect *bla TEM* gene (beta lactamase)which responsible for antibiotic resistance. The obtained results showed that 7 samples (5 cow and 2 buffalo) were positive for *P. aeruginosa* with percentage of (4. 1%).PCR results revealed that all 7 isolates were confirmed as *P. aeruginosa, oprl* gene was detected in all isolates, *toxA* and *exo S* genes were detected in 5 isolates. while *bla TEM* gene was detected in all isolates which indicate that *P. aeruginosa* is naturally resistant to beta lactam antibiotics.

Key words: *Pseudomonas aeruginosa, Bovine mastitis, PCR, virulence factors - bla TEM gene*

Introduction

Pseudomonas aeruginosa is an aerobic, motile, Gram-negative rod, widely present in the environment, water and in humid settings, but it is also an important opportunistic pathogen for humans, plants and animals (**Bergonier *et al.*, 2003)**.

*P. aeruginosa*is an environmental pathogen that can cause severe clinical mastitis with systemic signs as well as subclinical chronic mastitis. The organism is considered an opportunistic pathogen causing disease when the environment is contaminated and when the host defenses are decreased by stress, concomitant disease, or by nutritional imbalances (**Kirk and Bartlett, 1984)**.

Drug resistance and severe toxemia due to Pseudomonas infections are supposed to produce high case fatality rate in immune-suppressed host**.** There are many factors are responsible for *P. aeruginosa* infection in cattle and buffaloes, particularly exposure of open wounds with contaminated soils and water (**Geyik *et al.,* 2003)**.

Clinical mastitis resulting from herd infection of *P.aeruginosa* has been reported to be caused by a variety of factors, including contaminated dry-off preparations **(Osborne *et al.*, 1981)**, teat wipes (**Daly *et al.,* 1999; Power, 2003)**and wash water used to clean udders prior to milking (**Erskine *et al.,* 1987).**

Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors such as lipopolysaccharide (LPS), flagellum, and pilus and non-pilus adhesins, as well as to exoenzymes or secretory virulence factors, including protease, elastase, phospholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins, and siderophores **(Hentzer *et al.,* 2003)**.

P. aeruginosa possess several pathogenic fac-

tors respon-sible for their pathogenicity, among which exotoxin A (*tox*A) and exoenzyme S (*exo*S) are two major fatal weapons which remain associated with subclinical mastitis infection in bovines **(Narayanan, 2013)**.

L and I lipoproteins are two outer membrane proteins of *P. aeruginosa* responsible for inherent resistance of *P. aeruginosa* to antibiotics and antiseptics. As these proteins are found only in this organism, they could be a reliable factor for rapid identification of P. aeruginosa in clinical samples (**Nikaido, 1994) and (de Vos D *et al*., 1997) .**

P. aeruginosa has the ability of causing mastitis in dairy cow, this bacterium presents a difficult challenge, as it tends to protect itself from antibiotics and white blood cells in layers of slim (**Monett *et al.,* 2004).** It is highly resistant to many antibiotics and such phenomenon might be due to R-factor (**El- Jakee *et al.,* 1995).**

Polymerase chain reaction (PCR) has been developed to identify various mastitis pathogens (**Meiri-Bendek *et al.,* 2002)**. The development of PCR-based methods provides a promising option for the rapid identification of bacteria. With this method, identification of bacterial pathogens can be made in hours, rather than the days required for the conventional culture methods. Molecular methods have been reported to be superior to the phenotypic methods for identification of *P. aeruginosa* **(Qin *et al.,* 2003)**.

Therefore, this study was aimed for isolation and identification of *Pseudomonas aeruginosa* from milk samples collected from mastitic cow and buffalo in addition to application of PCR to confirm *P. aeruginosa* isolates, detect of some virulence genes that associated with pathogenicity (*toxA, exoS* and *oprl* genes) and for detection of *bla TEM* gene (beta lactamase) which responsible for resistance to beta lactam antibiotics.

Materials and Methods

Collection of samples: A total of 170 milk samples were collected from mastitic cow and buffalo (100 cow and 70 buffalo) from different localities at El-Behera governorate. The samples were collected, labeled and transported with a minimum of delay in ice box to the laboratory for bacteriological examination.

Isolation and Identification of *Pseudomonas aeruginosa*: The milk samples were plated onto *Pseudomonas* agar medium supplied with Cetrinix Supplement (Cetrimide and nalidixic acid) obtained from Hi Media (India) after overnight enrichment on nutrient broth followed byincubation aerobically at 37°C for 24 hrs. The suspected colonies were subjected to further morphological and biochemical identification according to **Kreig and Holt (1984).**

Molecular characterization of *Pseudomonas aeruginosa:* PCR was carried out using (16SrDNA of *P. aeruginosa*) to identify *P. aeruginosa*, detect of some virulence genes using specific primers to *toxA , exoS* and *oprl* genes **(Spilker *et al*., 2004 and Nikbin *et al.,* 2012)** and detect of *bla TEM* gene (beta lactamase) by using of specific primer to*bla TEM* gene.

DNA extraction. DNA extraction from isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at $56^{O}C$ for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primers:. Primers used were supplied from Metabion (Germany) are listed in table (1).

PCR amplification. For PCR, 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 were separated by elec-

trophoresis 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 PCR products were loaded in each gel slot. Gelpilot 100 bp DNA ladder (qiagen, gmbh, Germany) and Generuler 100 bp ladder (Fermentas, Thermo Scientific, 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 through computer software **Nikbin *et al.*, (2012)** PCR was carried out in the Reference lab. For veterinary quality control on poultry production (Egypt).

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
ToxA	GACAAC-GCCCTCAGCATCAC CAGC CGCTGGCCCATTCG CTCCAGCGCT	396	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Matar *et al.*, (2002)**
oprL	ATG GAA ATG CTG AAA TTC GGC CTT CTT CAG CTC GAC GCG ACG	504	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Xu *et al.*, (2004)**
exoS	GCGAGGTCAG-CAGAGTATCG TTCGGCGTCAC-TGTGGATGC	118	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Winstanley *et al.*, (2005)**
blaTEM	ATCAGCAATAAAC-CAGC CCCCGAAGAACGTT TTC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Colom *et al.*, (2003)**
P. aeruginosa 16S rDNA	GGGGGATCTTCG-GACCTCA TCCTTAGAG-TGCCCACCCG	956	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Spilker *et al.*, (2004)**

Results

1-Isolation of *Pseudomonas aeruginosa* from milk samples:

Seven milk samples (5 cows and 2 buffalo) were positive for *P. aeruginosa* with percentage of 4.1 % (7 isolates out of 170 samples) as in table (2).*P. aeruginosa* isolates showed fluorescent green color colonies on *Pseudomonas* agar platesphoto (1).The isolates were found to be Gram negative bacilli with Gram staining.

Table (2). Prevalence of *Pseudomonas aeruginosa* among the examined samples

Type of animal	No of examined samples	Positive samples	Percentage (%)
Cow	100	5	5%
Buffalo	70	2	2.8%
Total	170	7	4.1%

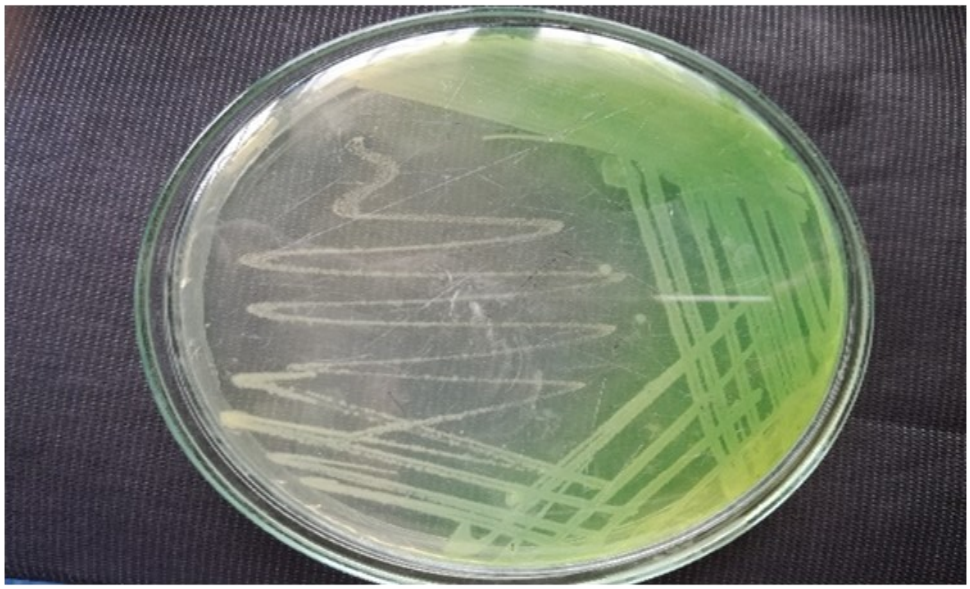

Photo. (1): Fluorescent green color colonies of *Pseudomonas aeruginosa* on *Pseudomonas* agar plates
2-Biochemical identification: The isolates showed positive to catalase, oxidese, nitrate reduction and citrate utilization test and were found negative to indole test, methyl red test and voges-Proskauer (VP) test.
3-Molecular characterization of *Pseudomonas aeruginosa:* The suspected seven isolates that biochemically characterized were subjected to PCR. The results are illustrated as in table (3) and photo (2- 6).

Table (3). Molecular characterization of *P. aeruginosa* and different detected genes.

isolate No	*Source of isolates*	*Tested genes*				
-	-	***P. aeruginosa 16S rDNA***	***toxA***	***exoS***	***oprL***	***blaTEM***
1	Cow	+	+	+	+	+
2	Cow	+	+	+	+	+
3	Cow	+	+	+	+	+
4	Cow	+	+	+	+	+
5	Buffalo	+	-	-	+	+
6	Buffalo	+	-	-	+	+
7	Cow	+	+	+	+	+

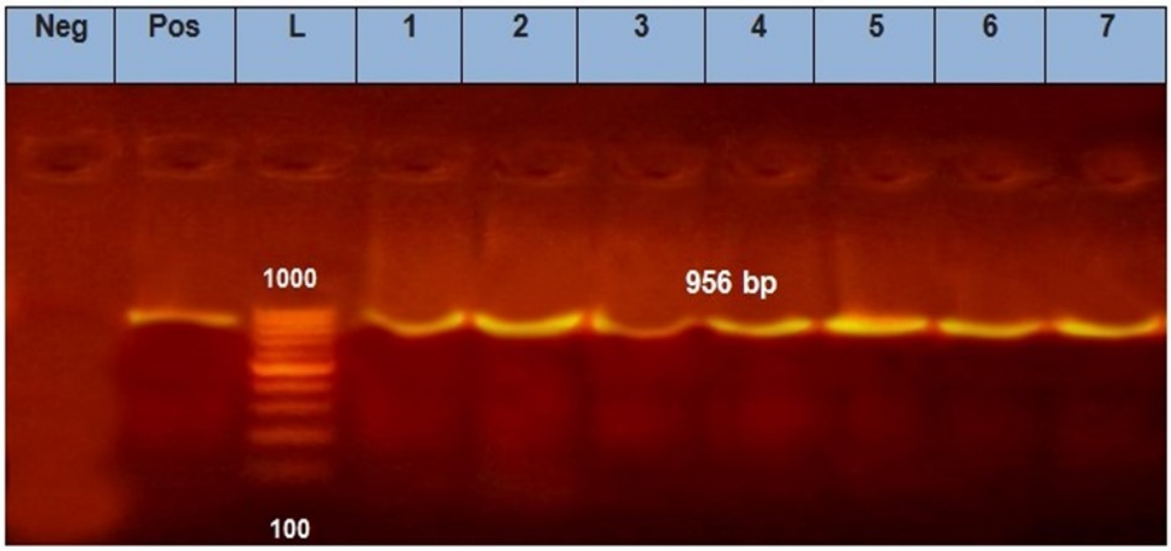

Photo. (2): Agarose gel electrophoresis of PCR products showing amplification of *P. aeruginosa* 16SrDNA gene products at 956 bp, lane L: molecular weight marker MWM (100 – 1000 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1, 2, 3, 4 and 7): *P. aeruginosa* isolated from cows, lanes (5, 6): *P. aeruginosa* isolated from buffalo. All isolates were positive to *P. aeruginosa*.

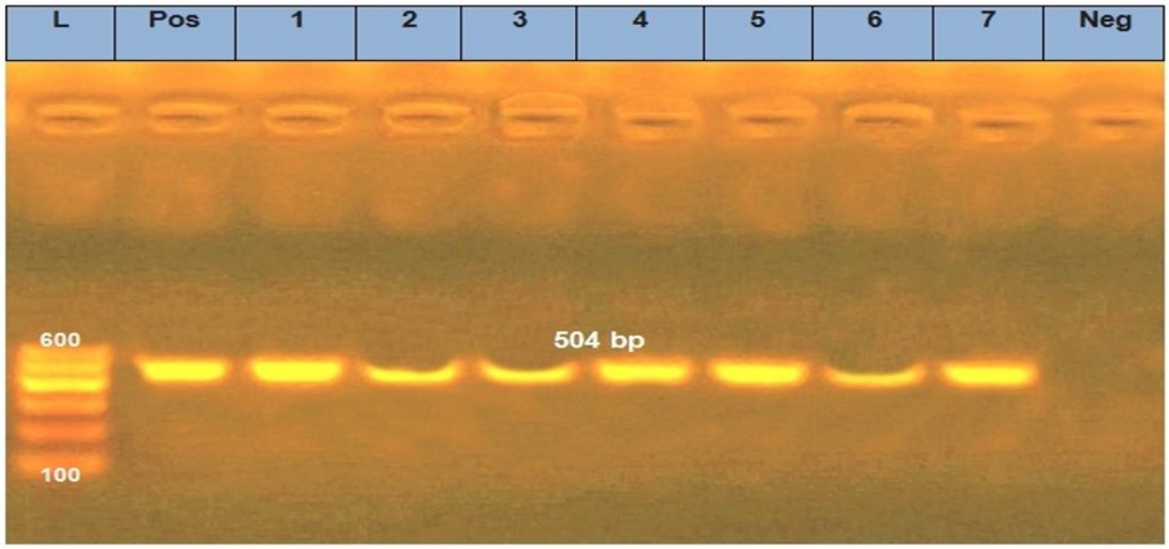

Photo (3) Agarose gel electrophoresis of PCR products after amplification of *oprL* gene products at 504 bp. lane L: molecular weight marker MWM (100 – 600 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1, 2, 3, 4 and 7): *P. aeruginosa* isolated from cows, lanes (5, 6): *P. aeruginosa* isolated from buffalo. All isolates were positive for *oprL* gene

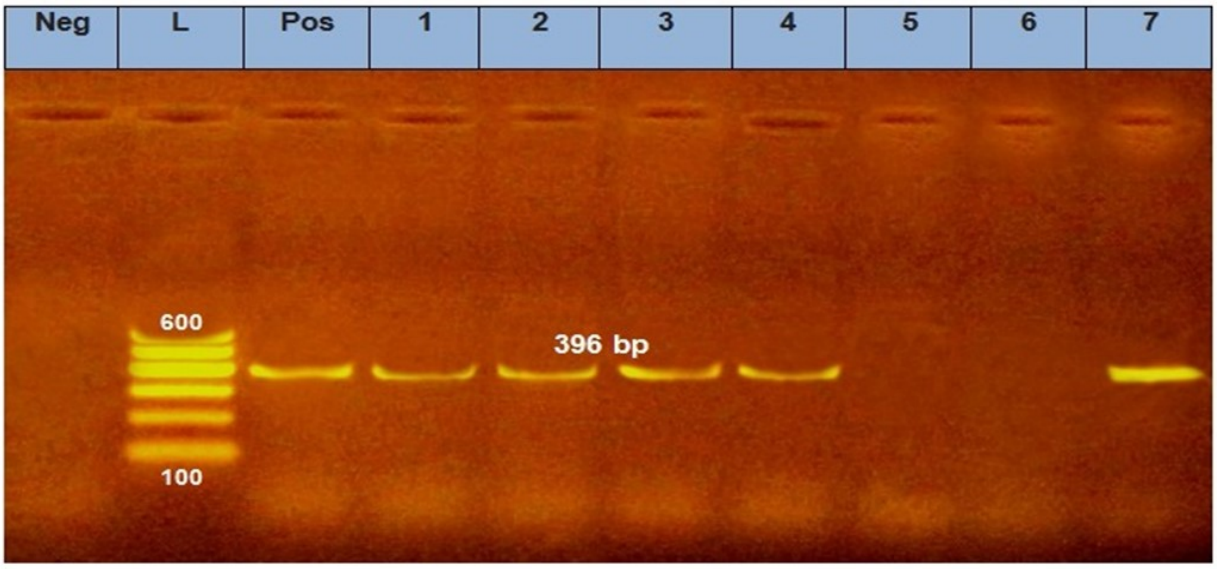

Photo. (4): Agarose gel electrophoresis of PCR products after amplification of *toxA* gene products at 396 bp. lane L: molecular weight marker MWM (100 – 600 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1, 2, 3, 4 and 7): *P. aeruginosa* isolated from cows, lanes (5, 6): *P. aeruginosa* isolated from buffalo. The isolates (1, 2, 3, 4 and 7) were positive for *toxA* gene while (5, 6) were negative.

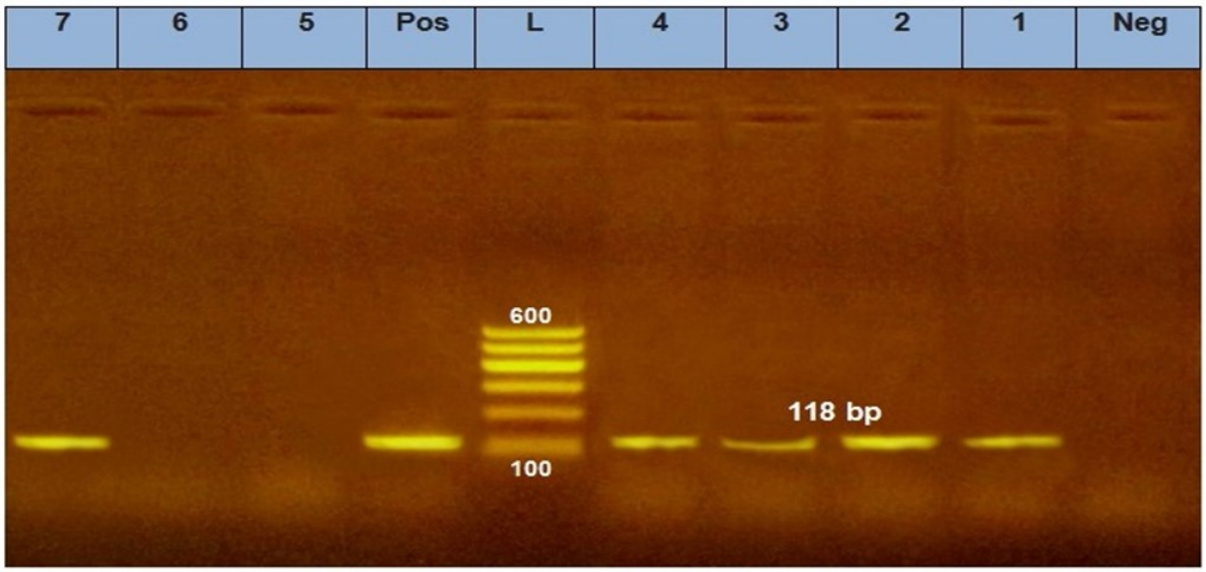

Photo. (5): Agarose gel electrophoresis of PCR products after amplification of *exoS* gene products at 118 bp. lane L: molecular weight marker MWM (100 – 600 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1, 2, 3, 4 and 7): *P. aeruginosa* isolated from cows, lanes (5, 6): *P. aeruginosa* isolated from buffalo. The isolates (1, 2, 3, 4 and 7) were positive for *exoS* gene while (5, 6) were negative.

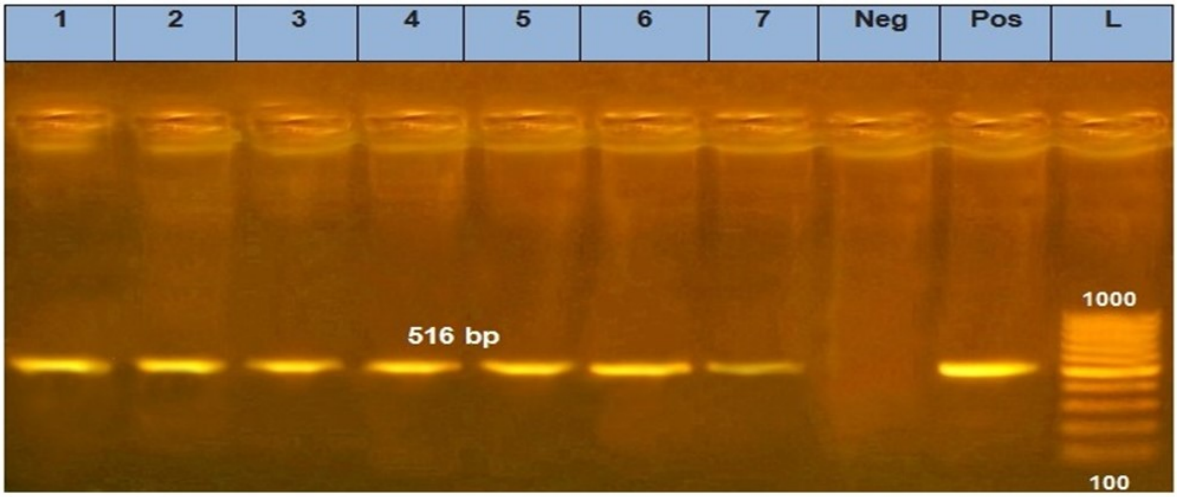

Photo. (6): Agarose gel electrophoresis of PCR products after amplification of *bla TEM* gene products at 516 bp**.** lane L: molecular weight marker MWM (100 – 1000 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1, 2, 3, 4 and 7): *P. aeruginosa* isolated from cows, lanes (5, 6): *P. aeruginosa* isolated from buffalo. All isolates were positive to *bla TEM* gene

Discussion

The incidence of clinical mastitis dueto *P. aeruginosa* has been increased in large dairy herds in recent years, causing large economic losses and significant problems for the affect edfarms. The origin of mastitis due to *P. aeruginosa* has been traced to contaminated water sources, with hoses and nozzles in milking parlors, and the pipes and tanks in cattle sheds, being the primary sources of infection (**Erskine *et al*., 1987; Hicks *et al*., 1991).**

P. aeruginosa has been reported to be among the three most prevalent cultured from bovine milk samples and the three most frequently associated with clinical mastitis (**Barkema *et al*., 1998).** *P. aeruginosa* produces two different toxins ETA (exotoxin A) and exoenzyme S **(Bodey *et al*., 1983)**. The highly toxic ETA is produced by the majority of *P. aeruginosa* strains and can inhibit eukaryotic protein biosynthesis at the level of polypeptide chain elongation factor 2, similarly to diphtheria toxin. ETA consists of two subunits; fragment A is catalytic, and fragment B is responsible for interaction with eukaryotic cell receptors. ETA is cytotoxic to numerous mammalian cells **(Matthew, 1983).** Exoenzyme S causes significant tissue damage in lung, burn and wound infections **(Wolfgang *et al*., 2003)**.

Table (2) showed that percentage of *P. aeruginosa* isolated from examined milk samples-was 4.1% (7 out of 170). These results were nearly similar to those obtained by **Al Dabbagh (2012)** who isolated *P. aeruginosa*4.4 % from clinical and subclinical mastitis in Mosul city. Lower incidence was recorded by **Sori *et al.,* (2005)** who isolated *P. aeruginosa* from clinical bovine mastitis with percentage of 2.13 % and **Sumathi *et al.,* (2008)** who isolated *Pseudomonas* spp. from clinical bovine mastitis with percentage of 1.33%. Moreover, **Atyabi *et al.,* (2006)** isolated *P. aeruginosa* from bovine mastitis with a percentage of ,21 %in Tehran. Higher isolation rate was be detected by **Younis *et al*., (2015)** who isolated *P. aeruginosa* from mastitic milk samples with percentage of 25%, **Ghazy *et al*., (2015)** isolated *P. aeruginosa* from bovine mastitis with percentage of 10 % and **Saleh *et al*., (2016**) who isolated *P. aeruginosa* with percentage of 11.4 % from milk of mastitic cattle .

The percentage of *P. aeruginosa* isolated from buffalo milk samples was 2.8% as in table (2), that approach to **Heleili *et al.,* (2012)**whofound that the prevalence of *Pseudomonas* spp. in sub -clinical mastitis cases of bovines and buffaloes was (**3.0**%). Moreover, **Baloch *et al.*, (2011)** found thatthe incidence of *P. aeruginosa* from clinical mastitis in buffalo was 1.42%.

Higher incidence detected by **Vishwakarma (2008)** who reported that the prevalence of *Pseudomonas* spp. in sub-clinical mastitis cases of bovines and buffaloes was 6.9%**.** The variation in incidences among authors could be attributed to the variation of hygienic conditions, managmental parameters and variations among the animal holders as well as sample volume.

Molecular characterizations of the isolates were done using (16SrDNA of *P. aeruginosa*) by conventional PCR. The results revealed that all seven isolates were confirmed as *P. aeruginosa* table (3) and photo (2).*P. aeruginosa* possesses a variety of virulence factors that may contribute to its pathogenicity. PCR was used for detection of *P. aeruginosa* exotoxin A (*toxA)*, exoenzyme S (*exo S)* and *oprL* gene. The obtained results demonstrated that *oprL* gene was detected in all seven isolates of *P. aeruginosa* photo (3), this agree with **Mohammed *et al.*, (2015)** who detected *oprL* gene in all examined isolates from raw milk (100 **%)** and **Nikbin *et al.*, (2012)** who detected *oprL* gene (L- lipoprotein) among all examined *P. aeruginosa* isolates from different infectious origins while **Neamah (2017)** detected *oprL* gene in examined milk samples with a percentage of 50**%**.

ToxA gene was detected in 5 isolates (1, 2, 3, 4, 7) photo (4). This matching with **Banerjee *et al*., (2017)** who found that the percentage of *toxA* gene in examined isolates from bovine subclinical mastitis was 63.2% while **Lanotte *et al*., (2004)** and **Mohammed *et al*., (2015)** detected*toxA gene* in all examined isolates with percentage of 100**%.** On the other hand **Younis *et al*., (2015)** could not detect *toxA gene* (0.00%) from examined milk samples but could detected *toxA gene* with percentage of 8.57% from other samples(nasal swabs and wound).

ExoS genes was detected in 5 isolates (1, 2,3, 4, 7) photo (5), this agree with **Sharma *et al.*, (2004), Badr *et al*., (2008) and Nikbin *et al.*, (2012)** who reported that the presence of *exoS* genes in examined isolates was 50-84**%** and **Neamah (2017)** who detected *exoS* genes with percentage of 75% from examined milk samples**.** While greater than **Younis *et al.*, (2015)** who detected *exoS* genes with percentage of 16% from examined milk samples and **Banerjee *et al.*, (2017**) who detected *exoS* genes with percentage of 36.8% while lower than **Lanotte *et al* ., (2004)** who detected *exoS* genes with percentage of 84.5%.

ToxA and *exoS* genes could not detected in isolates no (5**,** 6) which isolated from buffalo this may be matching with **Younis *et al.*, (2015)** as theycould not detect *toxA gene* from examined milk samples and this may be due to absence of expression enhancer gene of *toxA* (*PtxR* gene) from some isolates of *P. aeruginosa* as observed by **Vasil *et al.*, (1986**) who reported that the *PtxR* gene expression enhancer gene of *toxA* was only detected in *P. aeruginosa* isolates, whereas other species of *Pseudomonas* did not yield any positive results. Low sensitivity with *toxA* PCR screening is due to the fact that some isolates of *P. aeruginosa* do not carry this gene naturally.

Several mechanisms are involved in *P. aeruginosa* resistance to antimicrobial agents, such as chromosomal expression of resistance encoding genes, β-lactamase production, efflux pumps and decrease inmembrane permeability **Rodrigues *et al*., (2011**). PCR assays were developed with specific primers for the detection of *bla TEM* gene (beta lactamase). The results showed that *bla TEM* gene were detected in all seven isolates of *P. aeruginosa* photo (6), this indicate that all isolates resist to β-lactamantibiotic**.** This agree with **Saleh *et al*., (2016)** who detected beta lactamase (*bla – OX-A*gene) in all *P. aeruginosa* isolates from milk of mastitic cattle. While **Das *et al., (2017*)** detected *bla TEM* gene with percentage of (12%) from examined isolates of Gram negative bacteria including *pseudomonas* causing subclinical mastitis**.** The differences in prevalence of *P. aeruginosa* and distribution of its genes observed among different studies may be due to variation in hygienic standards of the dairy environment and milking conditions, management practices, different environmental and geographical cause, breeds of the animals, immune responses, climatic conditions as well as genetic variation in disease resistance amongst the breeds of animals.

Conclusion

Bovine mastitis associated with *Pseudomonas aeruginosa* has been increased in recent years. *P. aeruginosa* could beisolated from mastitic cow and buffalo. Virulence genes (*toxA, exoS* and *oprl* gene) and antibiotic resistance gene (*bla TEM* gene) were detected in *P. aeruginosa* isolates and this suggested that they are associated with different levels of intrinsic virulence and antibiotic resistance of *P. aeruginosa* isolates. From this study we recommended that hygienic measures and good management in dairy herds should be applicated, application of pre and post milking treatments, routine examination to water source in the farm for any pathogen especially *pseudomonas* and rapid dealing with immunologically depressed animals especially after viral infection to prevent *P. aeruginosa* infection causing large economic losses as it is an opportunistic pathogen and naturally resistant to wide spectrum of antibiotics.

References

Al-Dabbagh, S.Y.A. (2012). Bovine mastitis caused by gram negative bacteria in Mosul. Iraqi Journal of Veterinary Sciences. Vol. 26, No 1: 11-16.

Atyabi, N.; Vodjgani, M.; Gharagozloo and Fand Bahonar, A. (2006). Prevalence of bacterial mastitis in cattle from the farms around Tehran. Iranian Journal of Veterinary Research, University of Shiraz, Vol. 7, No. 3, Ser. No. 16.

Badr, H.A.R.; El-Nagdy, M.; El-Sabagh, A. and El-Din, A.B. (2008). *Pseudomonas aeruginosa* exotoxin a as a virulence factor in burn wound infections. Egypt. J. Med. Microbiol., 17(1): 125-132.

Baloch, H.; Rind, R.; Kalhoro D.H. and Kalhoro A.B. (2011). Study on the incidence of clinical mastitis in buffaloes caused by bacterial species. Pak. J. Agri., Agril. Engg., Vet. Sci., 27 (1): 83-93.

Banerjee, S.; Batabyal, K.; Joardar, S.N.; Isore, D.P.; Dey, S.; Samanta, I.; Samanta, T.K. and Murmu, S. (2017). Detection and characterization of pathogenic *Pseudomonas aeruginosa* from bovine subclinical mastitis in West Bengal, India, Veterinary World, 10 (7): 738-742.

Barkema, H.W.; Schukken, Y.H.; Lam, T.J.; Beiboer, M.L.; Wilmink, H.; Benedictus, G. and Brand, A. (1998). Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J. Dairy Sci. 81, 411–419.

Bergonier, D.; de Cremoux, R.; Rupp, R.; Lagriffoul, G. and Berthelot, X. (2003). *Mastitis* of dairy small ruminants. Vet Res.; 34: 689–716.

Bodey, G.P.; Bolivar, R.; Fainstein, V. and Jadeja L. (1983). Infections caused by *Pseudomonas aeruginosa*. Rev. Infect. Dis. 5(2): 279-313.

Colom, K.; PèrezJ, A.R.; Fernández-A.L.; Cisterna R. (2003). Simple and reliable multiplex PCR assay for detection of bla_{TEM},bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiology Letters 223 (2003) 147-151.

Daly, M.; Power, E.; Bjorkroth, J.; Sheehan, P.; O'Connell, A.; Colgan, M.; Korkeala, H. and Fanning, S. (1999). Molecular analysis of *Pseudomonas aeruginosa*: epidemiological investigation of mastitis outbreaks in Irish dairy herds. Appl. Environ. Microbiol. 65, 2723–2729.

Das, A.; Guha, C.; Biswas, U.; Jana, P.S.; Chatterjee, A.; Samanta, I. (2017). Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal, Veterinary World, 10(5): 517-520.

de Vos D.; Lim, A.; Pirnay, J.P.; Struelens, M.; Vandenveld, C.; Duinslaeger, L. (1997). Direct detection and identification of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane genes, *oprI* and *oprL*. J Clin Microbiol; 35: 1295-1299.

El-Jakee, J.; Gamal, E.; Hatem, M.E.; Farid, A.F. and Kamelia, M.O. (1995). Antibiogram pattern of some bacteria recovered from diarrheic calves in Egypt. J. Egypt. Vet. Med. Asoc. 55 (3): 721-737.

Erskine, R.J.; Unflat, J.G.; Eberhart, R.J.; Hutchinson, L.J.; Hicks, C.R and Spencer, S.B. (1987). Pseudomonas mastitis: Difficulties in detection and elimination from con-

taminated wash water systems. Journal of the American Veterinary Medical Association 191, 811–815.

Geyik, M.F.; Aldemir, M..; Hosoglu, S. and Tacyildiz. H.I. (2003). Epidemiology of burn unit infections in children. American Journal Infected Control 6: 342-6.

Ghazy, A.E.; Alkatsha, M.I.; Khaliel, S.A and Noseir M.E. (2015). Phenotypic and Genotypic Characterization of *Pseudomonas Aerogenosa* isolated from bovine mastitis. Alexandria Journal of Veterinary Scienc, 44: 80-85.

Heleili, N.; Ayachi, A.; Melizi, M.; Kassah, A.L. and Mamache, B. (2012). Prevalence of sub-clinical bovine mas-titis and the *in vitro* sensitivity of bacterial isolates in Batna governorate, East of Algeria. J. Anim. Sci. Adv., 2(6): 576-582.

Hentzer, M.; Wu, H.; Andersen, J.B.; Riedel, K.; Rasmussen, T.B.; Bagge, N.; Kumar, N.; Schembri, M.A.; Song, Z.; Kristoffersen, P.; Manefield, M.; Costerton, J.W.; Molin, S.; Eberl, L.; Steinberg, P.; Kjelleberg, S.; Hoiby, N. and Givskov, M. (2003). Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J. 22: 3803–3815.

Hicks, C.R.; Eberhart, R.J.; Erskin, R.J. and Spencer, S.B. (1991). Interactions between *Pseudomonas aeruginosa* and iodophor germicides in milking parlor udder wash water systems. Applied and Environmental Microbiology 57, 568–572.

Kerig, N.R. and Holt, J.G. (1984). Bergey, s Manual of Systemic Bacteriology. 8Th Edition. Williams and Wilkins, Baltimore, London.

Kirk, J.H. and Bartlett, P.C. (1984). Nonclinical *Pseudomonas aeruginosa* mastitis in a dairy herd. J Am Vet Med Assoc;184: 671–673.

Lanotte, P.; Watt, S.; Mereghetti, L.; Dartiguelongue, N.; Rastegar-Lari, A.; Goudeau, A. and Quentin, R. (2004). Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins. *J. Med. Microbiol.,* 53: 73-81.

Matar, G.M.; Ramlawi, F.; Hijazi, N.; Khneisser, I. Abdelnoor, A.M. (2002). Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. current microbiology Vol. 45 (2002), pp. 350 –354.

Matthew, P. (1983). The role of exotoxin A in *Pseudomonas disease* and immunity. Rev. Infect. Dis. 5(5): 979-984.

Meiri-Bendek, I.; Lipkin, E.; Friedmann, A.; Leitner, G.; Saran, A.; Friedman, S. and Kashi, Y. (2002). A PCR-based method for the detection of *Streptococcus agalactiae* in Milk. J. Dairy Sci., 85: 1717-1723.

Mohammed, G.M.O.; **Megahed A.A. and Nasr S.S. (2015).** Bacteriological and molecular detection of *pseudomonas* species from raw milk sold in Port-Said City markets. Egypt. J. Chem. Environ. Health, 1 (1): 986-1002

Monnet, D.L.; Mackenzie, F.M.; Lopezlozano, J.M.; Beyaert, A.; Camacho, M.; Wilson, R.; Stuart, D. and Gould, I.M. (2004). Antimicrobial drug use and methicillin-resistant *Staphylococcus aureus* Aberdeen, 1996-2000. Emerg. infect. Dis., 10(8): 1432-41.

Narayanan, S. (2013). Pseudomonas. In: McVey, D.S., Kennedy, M. and Chengappa, M.M., editors. Veterinary Microbiology. 3rd ed. John Wiley & Sons, Inc., New York. p148-150.

Neamah, A.A. (2017). Molecular detection of virulence factor genes in *Pseudomonas aeruginosa* isolated from human and animals in Diwaniya province. Kufa Journal for Veterinary Medical Sciences Vol. (8) No. (1) 218-230.

Nikaido, H. (1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science*; 264: 382–388.

Nikbin, V.S.; Aslani, M.M.; Sharafi, Z.; Hashemipour, M.; Shahcheraghi, F. and Ebrahimipour, G.H. (2012). Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. *Iran. J. Microbiol.*, 4(3): 118-123.

Osborne, A.D.; Armstrong, K.; Catrysse, N.H.; Butler, G. and Versavel, L. (1981). An outbreak of Pseudomonas mastitis in dairy cows. Can. Vet. J. 22, 215–216.

Power, E. (2003). Gangrenous mastitis in dairy herds. Vet. Rec. 153, 791–792.

Qin, X.; Emerson, J.; Stapp, J.; Stapp, L.; Abe, P. and Burns, L. (2003). Use of real-time PCR with multiple targets to identify Pseudomonas aeruginosa and other non fermenting gram-negative bacilli from patients with cystic fibrosis. J Clin. Microbiol. 4: 4312-4317.

Rodrigues, A.C.; Chang, M.R.; Nobrega, G.D.; Rodrigues, M.S.; Carvalho, N.C.; Gomes, B.G. (2011). Metallo-betalactamase and genetic diversity of *Pseudomonas aeruginosa* in intensive care units in Campo Grande, MS, Brazil. Braz J Infect Dis.;15 (3):195-9

Saleh, Z.F.; Mohamed, J. and Jawad, M.S. (2016). Isolation of *Pseudomonas aeruginosa* and molecular detection of bla-OXA gene of the bacteria from milk of mastitis cattle and from the wounds. AL-Qadisiya Journal of Vet. Med. Sci. Vol. 15 No. 1, 29-33.

Sharma, S.; Kaur, R.; Yadav, V.; Harjai, K. and Joshi, K. (2004). Contribution of exotoxin A of *Pseudomonas aeruginosa* in acute & chronic renal infection. Jpn. J. Infect. Dis., 57: 119-120.

Sori, H.; Zerihun, A. and Abdicho, S. (2005). Dairy Cattle Mastitis In and Around Sebeta, Ethiopia. Intern. J .Appl Res Vet Med • Vol. 3, No. 4, 332-338.

Spilker, T.; Coenye, T.; Vandamme, P. and LiPuma, J.J. (2004). PCR-Based Assay for Differentiation of *Pseudomonas aeruginosa* from Other *Pseudomonas* Species Recovered from Cystic Fibrosis Patients. journal of clinical microbiology, May 2004, p. 2074–2079.

Sumathi, B.R.; Veeregowda, B.M. and Amitha R.G. (2008). Prevalence and antibiogram profile of bacterial Isolates from clinical bovine mastitis Veterinary World, Vol.1(8): 237-238.

Vasil, M.L.; Chamberlain, C. and Grant C.C.R. (1986). Molecular studies *Pseudomonas* exotoxin A gene. Infect Immun; 52: 538-548.

Viswakarma, P. (2008). Studies on Prevalence, Diagnosis, Therapy and Control of Mastitis in Buffaloes. M. V. Sc. Thesis. Indira Gandhi Agricultural University, Raipur, Chhattisgarh.

Winstanley, C.; Kaye, S.B.; Neal, T.J.; Chilton, H.J.; Miksch, S.; Hart, C.A. and the Microbiology Ophthalmic Group (2005). Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. Journal of Medical Microbiology (2005), 54, 519–526.

Wolfgang, M.C.; Kulasekera, B.R.; Liang, X.; Boyd, D.; Wu, K.; Yang, Q.; Miyada, C.G. and Lory, S. (2003). Conservation of genome content and virulence determinants among cilinical and environmental isolates of *P. aruginosa.* Proceeding of the National Academy of Science of the United States of America. 100: 8484-9.

Xu, J.; Moore, J.E.; Murphy, P.G.; Millar, B.C. and Elborn, J.S. (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. 3:21.

Younis, G.; Awad, A.; Maghawry, M. and Selim, F. (2015). Extracellular enzymes and toxins of *Pseudomonas aeruginosa* strains isolated from clinically diseased Egyptian cows. Adv. Anim. Vet. Sci. 3(10): 522-526.