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A STUDY ON THE ROLE OF *KLEBSILLA PNEUMONIAE* IN MASTITIS IN SOME DAIRY FARMS

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ABSTRACT

Klebsiella pneumoniae is a common environmental pathogen which causes mastitis in dairy cows and is usually treated with antibiotics, thereby potentially increasing antimicrobial resistance. In the present study, 200 milk samples were collected from 160 mastitic cows, in addition to 40 apparently normal milk samples from in cows contact with mastitic ones as well as environmental samples (n = 44) from private dairy farms in Giza suffering from problems of mastitis and a decrease in milk production. Bacteriological examination revealed that *Klebsiella pneumoniae* was predominant in an incidence of (84.5%), *E. coli* (12.6%) and *Pseudomonas aeruginosa* (2.9%).

All *Klebsiella pneumoniae* isolates were subjected to disc diffusion sensitivity tests and all were resistant to Amikacin, Amoxicillin/Clavulanic Acid, Pencillin, and Tetracycline (100%), followed by Ampicillin (96.6%), Erythromycin, Tobramycin (86.2%) and Chloramphenicol (81.6%).

The highest sensitivity was to Kanamycin (100%) and Neomycin (97.7%). Virulence and antibiotic resistant genes of *Klebsiella pneumoniae* were detected by PCR and all the tested six isolates of *Klebsiella pneumoniae* were positive for *fimH*, and *kfu* in virulence genes but *magA* was detected in five isolates. Also antibiotic resistant genes *bla TEM* and *tetA* were detected in all isolates by PCR.

Keywords:

Klebsiella pneumoniae; mastitis; antimicrobial resistance;virulence genes.

INTRODUCTION

Klebsiella species are widely distributed in nature and represent the causative agent of many diseases affecting animals, man and birds. Also Klebsiella were found in a variety of environmental situations such as soil, water and vegetation rations **(Siu *et al.*, 2011)**.

Klebsiella pneumoniae is responsible for a variety of diseases in humans and animals **(Brisse *et al.*, 2006)**. Most notoriously, *K. pneumoniae* is a prominent nosocomial pathogen mainly responsible for urinary tract, respiratory tract or blood infections **(Podschun and Ullmann, 1998**).

Isolates from hospitals often display antibiotic resistance phenotypes **(Paterson *et al.*, 2004 and Woodford *et al*., 2007).**

Worldwide, mastitis is one of the most important and costly infectious diseases of the dairy industry, affecting animal welfare and having potential public health implications if untreated or if inadequately treated milk is consumed. In the etiology of bovine mastitis, Gram-negative organisms such as *Escherichia coli* and *Klebsiella pneumoniae* are regarded as significant agents of environment-associated bovine mastitis **(Bradley *et al.*, 2007).**

Klebsiella pneumoniae is a common cause of clinical mastitis in dairy cattle **(Roberson *et al.*, 2004 and Brisse and Duijkeren, 2005).**

Subclinical mastitis (SCM) is considered as the most important cause of economic losses that results in a marked reduction in quality and quantity of milk as well as it leads to lactation termination and involuntary culling of dairy buffaloes **(Shahid *et al.*, 2011).**

K. pneumoniae can multiply rapidly in bovine mammary tissues, causing a severe inflammatory response and tissue damage **(Bannerman *et al.*, 2004 and Cheng *et al.*, 2020)**.

Antibiotic resistance is a global public health problem and antibiotic resistant bacteria such as *Klebseilla pneumoniae* is common in the digestive tract and upper respiratory tract of animals and humans **(Sofiana *et al.*, 2020)**.

Antimicrobial resistance is commonly related to the spread of transmissible plasmids and the acquisition of resistance genes that normally occur by horizontal gene transfer, which may also carry virulence determinants **(Derakhshan *et al.*, 2016).**

The production of β-lactamase enzymes by the presence of β-lactam-insensitive cell wall transpeptidases, or the active expulsion of β-lactam molecules from Gram-negative bacteria represent the main indications of β-lactam antibiotic resistance **(Wilke *et al.*, 2005)**.

Klebsiella spp. are defined as germs that easily produce enzymes like extended-spectrum - beta lactamases (ESBL) which give them the ability to survive longer than other Gram-negative rods in the environment **(Livermore 1995)**.

Broad spectrum of virulence factors may lead to the infection of *K. pneumoniae* usually and play an important role for the causing of pneumonia, bloodstream infection and pyogenic liver

abscesses in mammals **(Newire *et al.*, 2013).**

Factors that are implicated in the virulence of *K. pneumoniae* strains include the capsular serotype, lipopolysaccharide, iron-scavenging systems, fimbrial and non-fimbrial adhesions **(Ma *et al.*, 2005 and Brisse *et al.*, 2006)**

Virulence *magA* (mucoviscosity-associated gene A) causes mucoviscosity and encodes capsular polymerase and found within genes specific for *K. pneumonia* **(Yeh *et al.*, 2010).**

Type 1 fimbriae are encoded by *fimH* gene, which can facilitate bacterial adhesion and biofilm formation **(Zamani and Salehzadeh 2018).**

The invasion of *K. pneumoniae* in domestic animals not only causes hazard in livestock production but also poses a potential threat to public health since these animals can act as the reservoir of multidrug-resistant *K. pneumoniae* strains. Although antibiotic therapy is a widely tool for the treatment of infections caused by *K. pneumoniae*, however, antibiotic resistance in pathogenic bacteria from food-producing animals and environmental sources is recognized as a global problem for public health **(White *et al.*, 2002).**

Both handling and consumption of products of these animals colonized by *K. pneumoniae* may be an important source of antibiotic-resistant *K. pneumoniae* of possible human health significance **(Davis *et al.*, 2015)**.

The aim of the present work was to study the prevalence rate of *K. pneumoniae* from clinical and subclinical mastitis, identify by traditional method and VITEK2 compact. Also, the presence of the virulence (*fimH, kfu* and *magA*) and drug resistance genes (*bla TEM* and *tet A*) genes which reflect the present of drug resistance in dairy farms to word the most used antibiotics were to be investigation.

MATERIAL AND METHODS

Samples:

In this study, 200 milk samples were collected from 160 mastitic cows, in addition to 40 apparently normal milk samples from contact animals. The Samples were collected from private dairy farms in Giza Governorate that suffering from problems of mastitis and a decrease in milk production throughout 2019.

All cows with clinical mastitis were examined on the basis of the inflammatory reaction and changes in the milk color of the affected quarters. Detection of subclinical mastitis cases was dependent on a positive California Mastitis Test with normal udder and milk color. The

samples were aseptically collected from the affected quarters before any antimicrobial treatment.

Prior to sampling, the udder was washed and dried and the teat was disinfected. Approximately 15 ml milk was collected into screw capped bottles and transferred to the laboratory on ice without dely.

The collected samples for bacteriological examination are depicted in (Table 1).

Table (1): Types of samples collected from dairy farms.

Types of samples	Number
Milk samples	**200**
Bulk milk	**12**
Swabs from milk machines	**12**
Water samples	**4**
Feed samples	**12**
Bedding	**4**
Total	**244**

Bacteriological examination:

Preparation of samples:

Milk samples were centrifuged for 15 min at 3000 rpm take loopful from sediment of each sample was inoculated into nutrient broth and incubated aerobically at 37^{o}C for 24 hr. A loopful from nutrient broth was streaked onto blood agar, MacConkey agar plates. The growing surface colonies were picked up, purified and re-inoculated into nutrient broth for further identification which was based on cultural, morphological and biochemical characteristics . All bacterial isolates were confirmed biochemically using traditional method reaction such as catalase, oxidase, in dole production, motility and citrate utilization. Biochemical reactions were carried out according to **McFadden (2000) and Quinn, *et al.*, (2011).**

Vitek2 Compact System biochemical Identification (Pincus, 2006).

Identification by Vitek2 compact system was done according to the manufacture's instruction (Biomeriux VITEK-2 Compact ref Manual – Ref-414532- France).

Antimicrobial sensitivity test:

Once they had been isolated and identified, pure cultures of Klebsiella isolates adjusted to McFarland tube No. 0.5, were tested against antibacterial with the disc diffusion assay on Mueller-Hinton agar. Testing was performed according to the recommendation of the Clinical and Laboratory Standards Institute **(CLSI 2017)**. The tested antibiotic discs were Amikacin (AK 30µg), Amoxicillin/ Clavulanic Acid (AMC 30µg), Ampicillin (Amp 15µg), Ceftriaxon (CRO 30µg), Cephalothin (KF30µg), Chloramphenicol (C 30µg), Doxycycline (DO 30 µg), Erythromycin (E 15µg), Gentamicin (GN 30µg), Kanamycin (K 30µg), Neomycin (N 30µg) Pencillin (P10 IU), Tetracycline (TE 30µg), Tobramycin (TOB 10µg), Trimethoprim-Sulphamethoxazol (SXT 25µg), and Vancomycine (VA30 µg) (Oxoid).

Detection of Some Virulence and Antibiotic Resistance Genes in *K. pneumoniae isolates*

DNA extraction. DNA extracted from six pooled isolates (one from each examined samples) of *Klebseilla pneumonia* were subjected to PCR assay for detection of some virulence genes and some drug resistance genes. Extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications of 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 absolute 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)** as listed in (Table 2).

Table (2): Primers sequences, target genes, amplicons sizes and cycling conditions for conventional PCR.

Target gene	Primers sequences 5/ --------3/	Amplified (bp)	Amplification (35 cycles)				Final extension	Reference
			Pre. denaturation	Sec. denaturation	Annealing	Extension		
TetA(A)	GGTTCACTCGAA CGACGTCA CTGTCCGACAAG TTGCATGA	576 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Randall *et al.* 2004
blaTEM	ATCAGCAATAAA CCAGC CCCCGAAGA ACGTTTTC	516 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Colom *et al.*, 2003
fimH	TGCAGAACGGA TAAGCCGTGG GCAGTCACCTG CCCTCCGGTA	508 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Ghanbarpo ur and Salehi, 2010
magA	GGTGCTCTTTA CATCATTGC GCAATGGCCAT TTGCGTTAG	1282 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 1.2 min.	72˚C 12 min.	Yeh *et al.*, 2007
Kfu	GAAGTGACGCT GTTTCTGGC TTTCGTGTGGC CAGTGACTC	797 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Osman *et al.*, 2014

PCR amplification. For PCR**,** primers were utilized in a 25- µl total volume containing 12.5 µl of Emerald Amp 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 electrophoresis on 1.5% agarose gel (Aleichem, 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. Gene ruler 100 bp ladder (Ferments, Thermo Scientific, Germany) was 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.

RESULTS AND DISCUSSION

Mastitis is the most costly and widespread infectious disease found in dairy farms. Antibiotic treatment is an indispensable tool used by dairy producers to prevent the occurrence of mastitis prior to calving and to cure persistent udder infections at the end of lactation. However, the development of antibiotic resistant bacteria is of increasing concern. In many instances, the indiscriminate use of antimicrobial agents in the treatment of mastitis in dairy cattle has increased the selection pressure towards antimicrobial resistance, **(Francis, 1989).** Economic losses due to mastitis include reductions in milk production, increased cost of production, reduced milk quality, reduced longevity, increased labor and treatment costs, and transmission to other animals **(Seegers *et al.*, 2003; Pinzón-Sánchez and Ruegg, 2011)**. Environmental mastitis means mastitis caused by bacteria that are transferred from the environment to the animal rather than from other infected quarters **(Radostits *et al.*, 1994).** *Klebsiella pneumoniae* is a common environmental agent of clinical and subclinical mastitis affecting dairy herds, and may be present in the final product decreasing its quality.

Mastitis caused by *K. pneumoniae* is even more severe due to its poor response to antibiotic therapy, rapid evolution to toxic shock and death of the animal **(Sampimon *et al.*, 2006 and Langoni *et al.*, 2015).**

In the present study the prevalence of *Klebsiella pneumoniae* (84.5%), was followed by *that of E. coli* (12.6%) and *P. Aeruginosa* (2.9%) as shown in (Table 3).That was in agreement with **those of Yasser (2010)** who isolated *Klebsiella pneumoniae* (85.7%). **Silva and Costa, 2001** isolated *K. pneumonia* with an incidence (54.5%). **Singh *et al.*, (2018**) recorded *Klebsiella pneumoniae*as (20.16%). **Ahmed *et al.*, (2016)** recoded that *Klebsiella pneumoniae* was (16.3%) while **Osman *et al.*, (2014)** mentioned that *Klebsiella pneumoniae* was (11.9%).

Fecal shedding of *Klebsiella* spp. contributes to pathogen loads in the environment, including milking machines, recycled manure, bedding material, alleyways, and holding pens (**Sampimon *et al.*, 2006, Zadoks *et al*., 2011, Schukken *et al.*, 2012)** *Klebsiella* is usually referred to as particularly aggressive and is prone to cause severe clinical mastitis, which responds poorly to treatment and as a consequence, infections tend to be severe and long lasting with a fatal outcome. **(Lucheis; 2012, Munoz *et al.*, 2007).** The existence of an oro-fecal transmission cycle has been suggested for *K. pneumoniae* in dairy herds, with fecal shedding resulting in the contamination of feed and water and the subsequent re-ingestion of

the organism, resulting in renewed fecal shedding. **Munoz *et al.*, 2007, Zadoks *et al.*, 2011 and Klaas and Zadoks, 2017).**

Studies have illustrated a correlation between bovine teat colonization by *Klebsiella* and the use of sawdust bedding **(Zdanowicz *et al.*, 2004).**

Confirmation of results of traditional identification by VITEK2 Compact system showed complete agreement with the results of traditional methods. A confirmatory identification by Vitek2 compact system was done using Gram negative card **(Biomeriux, Pincus, 2006)**.

The use of antibiotics inevitably selects both commensals and pathogenic bacteria. Human misuse of antibiotics has clearly put unnatural selective pressure on commensals and bacteria which has accelerated their evolutionary process towards resistance. The adaptation of microbes to the antibiotics increases with repeated use. Close election (same class) and cross selection (unrelated) to different antibiotics are exhibited by certain microbes along with direct selection **(O'Brien, 2002).**

Antibiotics are commonly used to treat bovine mastitis and consequently drug resistance in Gram-negative bacteria, including *K. pneumoniae,* is rapidly increasing. Therefore, it is important to identify alternatives to antibiotics for treating bacterial infections, especially for bovine mastitis caused by *K.pneumoniae* **(Saidani *et al.*, 2018 and Cheng *et al.*, 2019)**. The phenomenon of multidrug resistance (MDR) is defined as the ability of a cell to show resistance to a wide variety of structurally and functionally unrelated molecules **(Higgins, 2007).**

All isolates of *Klebseilla pneumonia* subjected to disc diffusion sensitivity test (Table 4) showed high drug resistance levels (100%) against Amikacin, Amoxicillin/Clavulanic Acid, Pencillin, and Tetracycline, followed by Ampicillin (96.6%), Erythromycin, Tobramycin (86.2%) and Chloramphenicol (81.6%). The highest sensitivity was to Kanamycin (100%) and Neomycin (97.7%). This almost agrees with **Dallal *et al.*, (2018),Walaa *et al.*, (2019)** recoded that *Klebseilla* spp. were resistant to Cefepime and Pipercillin /Tazobactam (84.6% each) and exhibited 100 % resistant to other antimicrobials agents. Meanwhile, all the recovered isolates were sensitive to imipenem and meropenem. **Osman *et al.*, (2014)** showed that *K.pneumoniae* were resistant to ampicillin (100%), chloramphenicol, colistin sulphate, erythromycin and streptomycin (88.9%), and penicillin (77.8%). **Cheng *et al*., (2018)** reported that *K. pneumoniae* isolates resistance rates to Ampicillin, Amoxicillin, Ciprofloxacin, Cefotaxime, Ceftriaxone, Gentamicin, Ceftazidime and Amikacin were 93.9%, 81.8%, 60.6%, 57.6%, 33.3%, 27.3%, 18.2% and 9.1%, respectively.

Genotyping is important to identify cases or outbreaks due to *K. pneumoniae* and to further track source and spreading of infections **(Guo *et al.*, 2015).**

Application of PCR for detection of virulence (*fimH* and *kfu* genes) was performed on six tested isolates of *K. pneumoniae* and all isolated showed amplicons of 248 bp and 797 bp respectively which as shown in (Table5) and photo (1).

PCR targeting *magA* gene showed amplicons of 1282bp in five tested isolates as shown in Table (5) and photo (2).

Osman *et al.*, (2014) showed that *magA* and *kfu*, rather than the K1 and K2 capsule *per se* are important virulence genes in invasive *K. pneumoniae* strains causing mastitis. Also *kfu* (iron uptake), *magA* (mucus viscosity) and *fimH* (fimbriae) had been detected in *Klebsiella pneumoniae* **(Jian-li *et al.*, 2017).** The *mag*A gene make the bacteria more invasive and more resistant to immune defense (phagocytosis) **(Soto *et al.*, 2012 and Shon *et al.*, 2013).** The *FimH* adhesion encoding genes, give an indication of their conservation in *K. pneumonia* pathogen **El-Fertas-Aissani (2013).**

In this study application of PCR for the detection of antibiotic – resistance genes (*blaTEM* and *tetA)* were done on six isolates which showed amplicons at 516 bp size and 576 bp respectively as shown in Table (5) and photo (3).

The extensive use of β-lactam antibiotics in cattle and other ruminants represent a considerable source for the transmission of antibiotic resistance genes (e.g., ESBL) or antibiotic resistant strains to the human intestinal bacterial flora **(Bandyopadhyay *et al.*, 2015).** ESBL producing *Klebsiella pneumoniae* was also isolated from the cases of bovine mastitis **(Locatelli *et al.*, 2010).**

Conclusion and recommendations:

K. pneumonia is a major cause of mastitis in both clinical and subclinical types as it leads to decreased milk production and subsequently salary loses. The virulence of organisms is due to presence of some virulence genes as *kfu, fimH* and *magA* which can be detected by PCR. Most of *K. pneumonia* isolates recovered in the study were highly resistance to most of the used antibiotics in the field a fined that may be attributed to the presence antibiotic resistance genes as *balATM* and *tetA.* Strict hygiene measures must be applied in the dairy farms.

Table (3): Prevalence of bacterial isolates from examined samples.

Samples	Bacteriologically positive samples		*E.coli*		*K.pneumonia*		*P. Aeruginosa*	
	NO	%	NO	%	NO	%	NO	%
Mastitic milk (160)	134	83.8	11	8.2	121	90.3	2	1.5
Apparently normal milk (40)	9	22.5	3	33.3	5	55.6	1	11.1
Bulk milk (12)	8	66.7	2	25.0	6	75.0	-	-
Swabs from milk machines (12)	10	83.3	2	25.0	6	75.0	2	20.0
Water samples (4)	3	75.0	1	33.3	2	66.7	-	-
Feed samples (12)	7	58.3	2	28.6	5	71.4	-	-
Bedding (4)	3	75.0	1	33.3	2	66.7	-	-
Total (244)	174	70.9	22	12.6	147	84.5	5	2.9

Table (4): Antimicrobial susceptibility of isolates *Klebseilla pneumoniae* (N.87).

Antibiotic (Ab)	Resistant isolates		Sensitive isolates	
	No	%	No	%
Amikacin (AK)	87	100	0	0
Amoxicillin/Clavulanic Acid (AMC)	87	100	0	0
Ampicillin (Amp)	84	96.6	3	3.4
Ceftriaxon (CRO)	52	59.8	35	40.2
Cephalothin (KF)	69	79,3	18	20.7
Chloramphenicol (C)	71	81.6	16	18.4
Doxycycline (DO)	65	74.7	22	25.3
Erythromycin (E)	75	86.2	12	13.8
Gentamicin (GN)	53	60.9	34	39.1
Kanamycin (K)	0	0	87	100
Neomycin (N)	2	2.3	85	97.7
Pencillin (P)	87	100	0	0
Tetracycline (TE)	87	100	0	0
Tobramycin (TOB)	75	86.2	12	13.8
Trimethoprim/Sulphamethoxazole (SXT)	55	63.2	32	36.8
Vancomycine (VA)	63	72.4	24	27.6

Table (5): Detection of virulence and anti-drug resistance genes in six *Klebseilla pneumonia* isolates.

***K. pneumonia* samples**	***blaTEM***	***TetA(A)***	***fimH***	***Kfu***	***magA***
1	+	+	+	+	-
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+

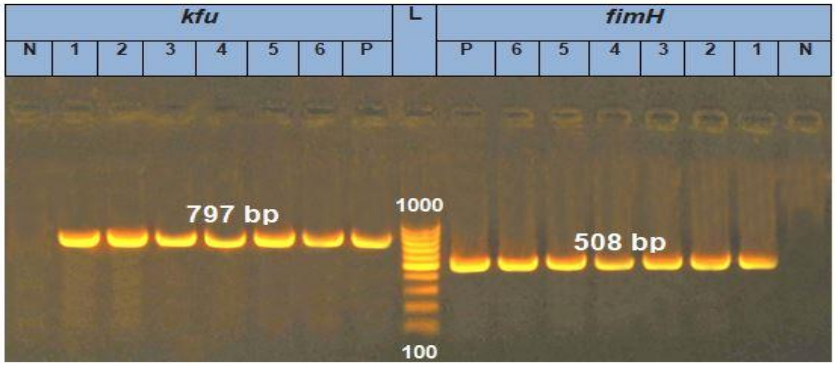

Photo. (1): Agarose gel electrophoresis of PCR products of *Klebseilla pneumoniae kfu* gene Lane L 100–1000 bp DNA ladder. N: negative control; P: positive control 797 bp. Lanes (1–6) positive to *kfu* gene. Amplification of *fimH* gene at 508bp amplified product. Positive isolates at 508bp. Lane L. Neg: Negative control Pos: positive control 508pb.Lane (1-6) were positive.

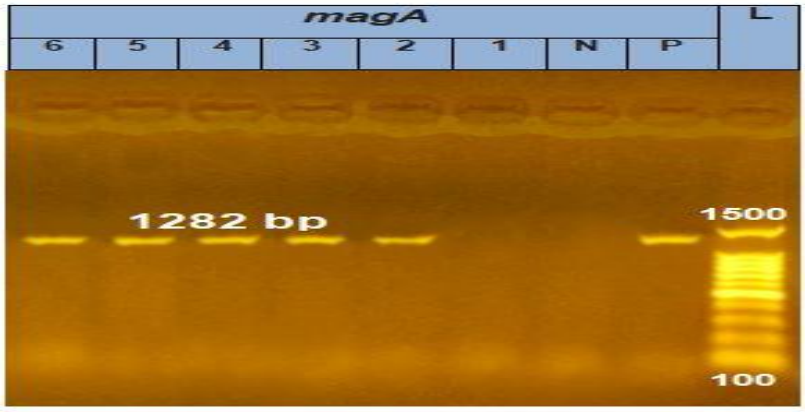

Photo. (2): Agarose gel electrophoresis of PCR products of *Klebseilla pneumoniae magA* Lane L 100 -1500bp DNA ladder. N: negative control; P: positive control. 1282bp.Lanes (2–6) positive and (1) negative.

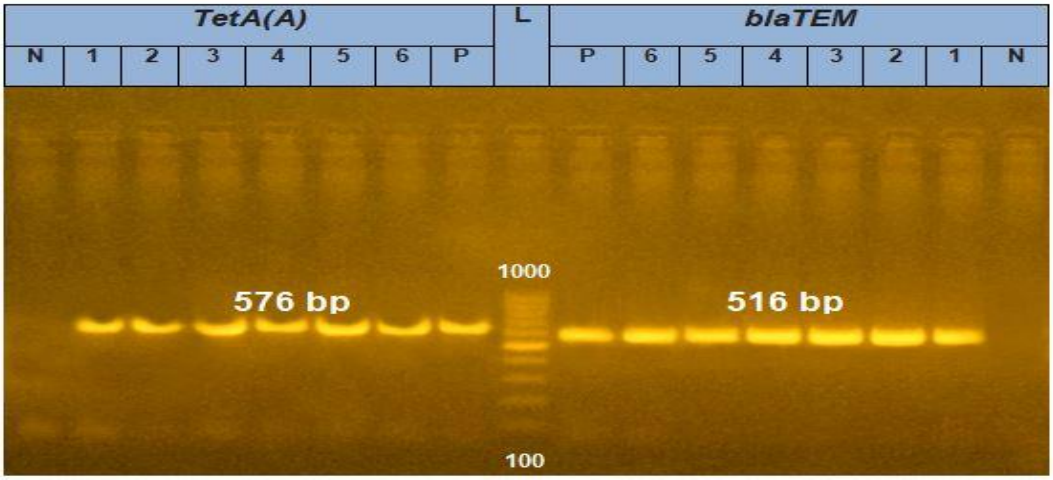

Photo (3): Agarose gel electrophoresis of PCR products of *Klebseilla pneumoniae blaTEM.* Lane L 100–1000 bp DNA laddering: negative control; P: positive Lanes (1–6) positive to *bla TEM* gene. Amplification of *tetA* gene at 576bp amplified product. Lane L: 100-1000 pb DNA ladder. Neg: Negative control Pos: positive control 576pb.Lane (1-6) positive.

REFERENCES

Ahmed, H.F. Hanaa, Asfour, A.E. Azza, M.K. Sobeih, Samah, F. Darwish, Mohamed Abu El-Magd, S.A. Darwish and Hanaa I.A. El -Saka (2016): Phenotypic and Molecular Identification of *Klebsiella* and *Salmonella* Species Isolated from Subclinical Mastitis Milk of Egyptian Buffalo Global Veterinaria 16 (5): 500-507, 2016.

Bandyopadhyay S, Samanta I, Bhattacharyya D, Nanda P.K, Kar D, Chowdhury J, Dandapat P, Das A.K, Batul N, Mondal B, Dutta T.K, Das G, Das B.C, Naskar S, Bandyopadhyay U.K, Das S.C, Bandyopadhyay S.(2015): Co-infection of methicillin-resistant *Staphylococcus epidermidis* methicillin-resistant *Staphylococcus aureus* and extended spectrum β-lactamase producing *Escherichia coli* in bovine mastitis - Three cases reported from India. Vet. Q. 2015; 35: 56–61.

Bannerman DD, Paape M, Hare WR, Hope JC. (2004): Characterization of the bovine innate immune response to intramammary infection with Klebsiella pneumoniae. J Dairy Sci. 2004; 87: 2420–32.

Bradley AJ, Leach KA, Breen JE, Green LE, Green MJ. (2007): Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet. Rec. 160:253–258.

Brisse S, Grimont F, Grimont PAD. The genus *Klebsiella*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E. (2006): editors. The *Prokaryotes · A* Handbook on the Biology of Bacteria. 3rd edition ed. New York: Springer; 2006.

Brisse, S. and van Duijkeren, E. (2005): Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. Vet. Microbiol., 105 (2005), pp. 307-312.

Cheng, F. Li a, Z. Lana, S. Liua, W. Li a, X. Zhoua, Z. Wua, Z.S. Zhang, M. Shan W. (2018): Characterization of Klebsiella pneumoniae associated with cattle infections in southwest China using multi-locus sequence typing (MLST), antibiotic resistance and virulence-associated gene profile analysis brazilian journal of microbiology 4 9S (2 0 1 8) 93-100.

Cheng J, Qu W, Barkema HW, Nobrega DB, Gao J, Liu G, De Buck J, Kastelic JP, and Sun H, Han B. (2019): Antimicrobial resistance profiles of 5 common bovine mastitis pathogens in large Chinese dairy herds. J Dairy Sci. 2019; 102: 2416-26.

Cheng J, Zhang J, Han B, Barkema HW, Cobo ER, Kastelic JP, Zhou M, Shi Y, Wang J, Yang R, Gao J. (2020) Klebsiellapneumoniae isolated from bovine mastitis is cytopathogenic for bovine mammary epithelial cells. J Dairy Sci. 2020; 103: 3493-504.

CLSI: (Clinical and Laboratory Standards Institute) (2017): Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Forth Informational Supplement; M100-27; CLSI: Wayne, PA, USA, 2017.

Colom K, Pèrez J, Alonso R, Fernández-Aranguiz A, Lariño E, 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.

Davis GS, Waits K, Nordstrom L. (2015): Intermingled Klebsiellapneumoniae populations between retail meats and human urinary tract infections. Clin Infect Dis. 2015; 61(6):892–89.

Derakhshan, S., Peerayeh, S. N., and Bakhshi, B. (2016): Association between presence of virulence genes and antibiotic resistance in clinical *Klebsiellapneumoniae* isolates. *Lab. Med.* 47, 306-311.

Dallal, M.M.S.; Khalifeh-Gholi, M.; Yazdi, S.S., Mehrani, F.; Hadayatpour, A. and Yazdi, M.K.S. (2018): Antimicrobial resistance pattern of Klebsiella spp isolated from patients in Tehran, Iran. J Surg Med, 2(2): 84-86.

El- Fertas-Aissani, R.; Messai, Y.; Alouache, S.; Bakour, R. (1013): Virulence profiles and antibiotic susceptibility patterns of Klebsiellapneumoniae strains isolated from different clinical specimens. Pathol. Biol. 2013, 61, 209–216.

Francis P.G. (1989): Update on mastitis. III. Mastitis therapy. *Br Vet J* 145: 302-11.

Ghanbarpour and Salehi (2010): Determination of Adhesin Encoding Genes in *Escherichia coli* Isolates from Omphalitis of Chicks. American Journal of Animal and Veterinary Sciences 5 (2): 91-96, 2010.

Guo C, Yang X, Wu Y, et al. (2015): MLST-based inference of genetic diversity and population structure of clinical *Klebsiella pneumonia* China. *Sci Rep*. 2015; 5: 7612.

Higgins C.F. (2007): Multiple molecular mechanisms for multidrug resistance transporters. *Nature* 446: 749–57.

Jian-li, W., Yuan-yuan, S., Shou-yu, G., Fei-fei, D., Jia-yu, Y., and Xue-hua, W., Yong-feng, Z., Shi-jin, J., and Zhi-jing, X. (2017): Serotype and virulence genes of Klebsiellapneumoniae isolated from mink and its pathogenesis in mice and mink. *Scientific Reports*, 7(1), 1-7.

Klaas I. C. Zadoks R. N. (2017): An update on environmental mastitis: Challenging perceptions. First published: 30 October 2017 https://doi.org/10.1111/tbed.12704.

Langoni, H. Vinicius, M. S. Guiduce, Diego B. Nóbrega, Rodrigo C. da Silva, Virgínia B. Richini-Pereira,Salina,A. and GuimarãesPesq. F.F.(2015): Researchof Klebsiellapneumoniae in dairy herds1 Vet. Bras. 35 (1):9-12, janeiro 2015.

Livermore, D.M. (1995): Beta-lactamases in laboratory and clinical resistance. Clinical microbiology reviews, 8: 557-584.

Locatelli C, Licia S, and Giuliano P, Paolo M. (2010): CTX-M1 ESBL-producing *Klebsiella pneumoniae* sub sp. *pneumoniae* isolated from cases of bovine mastitis. *J. Clin. Microbiol.* 2010; 48:3822.

Lucheis SB. (2012): Epidemiological and molecular characteristicsof *Klebsiella pneumoniae* infection in bovine dairy herds. São Paulo, SP: Agência Paulista de Tecnologia dos Agronegócios (APTA). *Secretaria de Agricultura e Abastecimento.* 2012.

MacFaddin. (2000): Biochemical tests for identification of medical bacteria. ”3rd edition". The Williams and Wilkins Baltimor. USA. Philadelphia.

Ma LC, Fang CT, Lee CZ, Shun CT, Wang JT (2005) Genomic heterogeneity in *Klebsiella pneumoniae* strains is associated with primary pyogenic liver abscess and metastatic infection. J Infect Dis 192: 117–128.

Munoz MA, Welcome FL, Schukken YH, Zadoks RN. (2007): Molecular epidemiology of two *Klebsiella pneumonia* mastitis outbreaks on a dairy farm in New York State. *J ClinMicrobiol.* 2007; 45: 3964 -71.

Newire, E.A. Ahmed, S.F. House, B. (2013): Detection of new SHV-12 SHV-5 and SHV-2a variants of extended spectrum beta-lactamase in *Klebsiella pneumoniae* in Egypt. Ann Clin Microbiol Antimicrob, 12 (2013), p. 16.

O’Brien TF. (2002): Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin Infect Dis 34(3):78–84

Osman, K.M.; Hassan, H.M.; Orabi, A. and Abdelhafez, A.S.T. (2014): Phenotypic, antimicrobial susceptibility profile and virulence factors of Klebsiellapneumoniae isolated from buffalo and cow mastitic milk. Pathogens and Global Health; 108. (4) 191-199.

Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, (2004): International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. *Ann Intern Med.* 2004; 140:26–32.

Pincus, D. H. (2006): Microbial identification using the biome'rieux VITEK® 2 System. Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association.

Pinzón-Sánchez, C. and Ruegg P.L. (2011): Risk factors associated with short-term post-treatment outcomes of clinical mastitis

Podschun R, Ullmann U. (1998): *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998; 11:589–603.

Quinn, P.J., B.K. Markey, F.C. Leonard, E.S. Fitz Patrick, S. Fanning and P.J. Hartigan, (2011): Veterinary microbiology and microbial disease. 2ed., wiley- ndblackwell, jwiley and sons ltd publication, UK.

Radostits OM; Blood DC; Gay CC. (1994): Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses. Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses, Ed. 8: xxiii + 1763 pp.; many ref.

Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V. and Woodward, M.J. (2004): Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy*.**53**, 208–216.

Roberson, J.R. Warnick, L.D. Moore G. (2004): Mild to moderate clinical mastitis: Efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment J. Dairy Sci., 87 (2004), pp. 583-592

Saidani M, Messadi L, Soudani A, Daaloul-Jedidi M, Châtre P, Ben Chehida F, Mamlouk A, Mahjoub W, Madec J, Haenni M. (2018): Epidemiology, antimicrobial resistance, and extended spectrum beta-lactamase-producing enterobacteriaceae in clinical bovine mastitis in Tunisia. Microb Drug Resist. 2018; 24:1242–8. 18. Díaz-Muñoz SL, Koskella B.

Sampimon OC; Sol J; Kock PA. (2006): An outbreak of *Klebsiella pneumoniae* mastitis. (Eenuitbraak van Klebsiella pneumoniae mastitis.) Tijdschriftvoor Diergenees kunde, 131(1): 2-4.Sci Rep, 5 (2015), p. 7612.

Schukken Y, Chuff M, Moroni P, Gurjar A, Santisteban C, Welcome F, *et al.* (2012): The 'other' Gram-negative bacteria in mastitis: *Klebsiella*, *serratia*, and more. *Vet Clin North Am: Food AnimPrac.* 2012; 28:239–56.

Seegers, H. Fourichon, C. Beaudeau, F. (2003): Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res., 34 (2003), pp. 475-49.

Seidler RJ. (1981): The genus *Klebsiella* (Nonmedical aspects). In: Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG, editors. *The Prokaryotes: a handbook on habitats, isolation, and identification of bacteria.* Berlin: Springer-Verlag; 1981. pp. 1166–1172.

Shahid, M., N. Sabir, I. Ahmed, R.W. Khan, M. Irshad, M. Rizwan and S. Ahmed (2011): Diagnosis of subclinical mastitis in bovine using conventional methods and electronic detector. ARPN. J. Agric. Biol. Sci., 6: 18-22. 3

Shon AS, Bajwa RP, Russ TA. (2013): Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence.* 2013; 4:107-18.

Silva N., Costa G.M.(2001): An outbreak of acute bovine mastitis caused by *Klebsiellapneumoniae* in a dairy herd Arq. Bras. Med. Vet. Zootec. vol.53 no.4 Belo Horizonte Aug. (2001).

Singh, K. Chandra, M. Kaur, G. Narang, D. Gupta, D.K. (2018): Prevalence and Antibiotic Resistance Pattern among the Mastitis Causing Microorganisms Journal of VeterinaryMedicine.Vol.08 No. 04 (2018).

Siu, L.K. Fung C.P., Chang F.Y. (2011): .Molecular typing and virulence analysis of serotype K1 *Klebsiella pneumoniae* strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong Singapore, and Taiwan

Sofiana Eka Dian , Junianto Wika Adi Pratama, Mustofa Helmi Effendi, Hani Plumeriastuti, Freshindy Marissa Wibisono, Erwan Budi Hartadi,Akvyan Rafi Hidayatullah (2020): A Review of the Presence of Antibiotic Resistance Problems on KlebsiellaPneumoniae Acquired from Pigs: Public Health Importance Sys Rev Pharm 2020;11(9):535-543.

Soto E, LaMon V, Griffin M, and Keirstead N, Palmour R. (2012): Phenotypic and genotypic characterization of *Klebsiellapneumoniae* isolates recovered from nonhuman primates. *J Wildlife Dis.* 2012; 48:603-11.

Walaa Gaffer, MayadaGwida, and Randa Abo Samra, Maha Al- Ashmawy (2019): extended spectrum beta-lactamase producingoccurrence and molecular characterization of *entero bacteriaceae*in milk and some dairy products Slov Vet Res 2019; 56 (Suppl 22): 475-85.

White DG, Zhao SH, Simjee S. (2002): Antimicrobial resistance of foodborne pathogens. Microbes Infect. 2002; 4:405-414.

Wilke, M. S., Lovering, A. L., and Strynadka, N. C. J. (2005): Beta-lactam antibiotic resistance: a current structural perspective. *Curr. Opin. Microbiol.* 8, 525–533.

Woodford N, Reddy S, Fagan EJ, Hill RL, and Hopkins KL. (2007): Wide geographic spread of diverse acquired AmpC beta-lactamases among Escherichia coli and *Klebsiella spp.* in the UK and Ireland. J Antimicrob Chemother. 2007; 59:102-105.

Yasser H.S. (2010): Some bacteriological studies on *Klebsiella pneumoniae* antigen in mastitic cattle. Ph.D Thesis. Cairo Univeristy, Faculty of Veterinary Medicine.

Yeh KM, Lin JC, Yin FY, Fung CP, Hung HC, Siu LK, Chang FY. (2010): Revisiting the importance of virulence determinant magA and its surrounding genes in *Klebsiella pneumoniae* causing pyogenic liver abscesses: exact role in serotype K1 capsule formation. J. Infect. Dis. 201:1259-1267.

Yeh, K.; Kurup, A.; Siu, L.K.; Koh, Y.L.; Fung, C.; Lin, J.; Chen, T.; Chang, F. and Koh, T. (2007): Capsular Serotype K1 or K2, Rather than *magA* and *rmpA*, Is a Major Virulence Determinant for *Klebsiella pneumonia* Liver Abscess in Singapore and Taiwan. JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2007, p. 466–471.

Zadoks RN,Middleton JR, McDougall S, KatholmJ,SchukkenYH.(2011): Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *J Mammary Gland BiolNeoplasia.* 2011; 16:357–72.

Zamani H and Salehzadeh A. (2018): Biofilm formation in uropathogenic Escherichia coli: association with adhesion factor genes. Turkish journal of medical sciences. 2018 Feb 23; 48 (1):162-167.

Zdanowicz M, Shelford JA, Tucker CB, Weary DM, Von Keyserlingk MA. (2004): Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *J Dairy Sci.* 2004; 87: 1694-701.

دراسة عن دور الكلبسيلا الرئوية فى التهاب الضرع فى بعض المزارع الحلابة

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الملخص العربى

كلبسيلا نيمونى أحد مسببات الأمراض البيئية الشائعة يسبب التهاب الضرع في الأبقار الحلابة وعادة ما يعالج بالمضادات الحيوية وبالتالي يحتمل أن يزيد من مقاومة مضادات الميكروبات. في هذه الدراسة تم تجميع 200 عينة حليب عبارة عن 160 من الأبقار المصابة ، بالإضافة إلى 40 عينة لبن من ابقار سليمة ظاهريًا كانت ملامسة لابقار مصابة بالتهاب الضرع وكذلك بعض العينات البيئية 44 من مزارع الألبان الخاصة في الجيزة التي تعاني من مشاكل التهاب الضرع وانخفاض في إنتاج الحليب. أظهر

الفحص البكتريولوجي أن عزل الكلبسيلا الرئوية التى كانت الأكثر انتشارا (84.5٪) والإشريكية القولونية (12.6٪) و كان السودوموناس (2.9٪). خضعت جميع المعزولات من كلبسيلا نيمونى لاختبارات الحساسية وكانت جميعها مقاومة للأميكاسين والأموكسيسيلين / حمض الكلافولانيك والبنسلين والتتراسيكلين (100٪) ، يليها الأمبيسلين (96.6٪) والإريثروميسين والتوبراميسين (86.2٪) لكل منهما ، بينما وكان الكلورامفينيكول (81.6٪). وكانت أعلى حساسية للكاناميسين (100٪) والنيومايسين (97.7٪). وباجراءانزيم البلمرة المتسلسل للجينات المقاومة و الجينات المقاومة للمضادات الحيوية لكلبسيلا نيمونى ان جميع المعزلات المختبرة (6) من لكلبسيلا نيمونى كانت إيجابية لـجين *fimH* ، وجين *kfu* ولكن جين *magA* في (5) معزولات. كما تم الكشف عن الجينات المقاومة للمضادات الحيوية *BlaTEM* و *tetA* في المعزولات المختبرة (6) كانت جميعها ايجابية.