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`VIRULOTYPING OF *KLEBSIELLA* SPP. *RECOVERED* FROM CAMEL CARCASSES

By

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ABSTRACT

The virulence of *Klebsiella species* were evaluated through both phenotypic and molecular assays. Virulence were assessed by the heamagglutination pattern, mannose resistance heamagglutination of *klebsiella* species against erythrocytes of different species (Cattle, sheep, horse and human (group O).Vero cell cytotoxicity, Suckling mouse assay and Congo red.

44 *klebsiella* isolates were recovered from internal organs of 150 camel carcasses with an overall incidence 29.3% .The sample were collected from slaughtered house in El-Kaliobia and El-Monofia governorates. The biochemical identification reavealed that 12 isolates were *k.pneumoniae*, 26 *k .oxytoca*, 6 *k. rhinoscleromatis*. The mannose resistant haemagglutination pattern of *k. pneumoniae* isolates against erythrocytes of cattle, sheep, horse and human (group O) were 7,8,5,6 respectively, while they were 3,2,4,4 for *k .rhinoscleromatis* and 18,15,18,14 *k. oxytoca*. Cytotoxicity of isolated strains to vero cells were 72.7 %, cells showing severe vaculation and detachment**.**12 isolates of *k. pneumonia* and 12 isolates of *k. oxytoca* screened via PCR for four virulence genes encoding fimbrial adhesins; *fim*H, invasins; *tra*T and *mag*A, and siderophores; *iut*A. All the 24 strains were positive to *fim*H and *tra*T amplified 508 and 300 bp respectively, while 23 of 24 strains harbor *iut*A and all 24 isolates don't possess *mag* A.

Keywords:

Klebsiella, Camels, heamagglutination, cell cytotoxicity, Virulence genes

INTRODUCTION

Genus *Klebsiella* is a Gram-negative, facultative anaerobic, non-motile bacillus. It is one of the *F. Enterobacteriaceae* causing infections, septicemia, pneumonia and urinary tract infections. The invasive syndrome is characterized by primary bacteremic liver abscesses and may associated with complications such as meningitis, endophthalmitis.*Klebsiella pneumoniae* mainly causing respiratory disorder and prolonged cough in camels **(Braiteh and Golden, 2007).** Presence of pulmonary lesions in apparently healthy camels prompted us to identify pathogenic agents that are associated with pneumonia in this neglected animal species. Under stressful conditions, bad sanitation, and immunosuppression, they could predispose camels to other infections. Middle East Respiratory Syndrome (MERS) is posited to be a zoonotic disease; Camels are essential to the continuous transmission of infection to humans **(Alagaili*et al.,*2014).** *K. pneumoniae* has been isolated from acute destructive bronchopneumonia and community acquired bacterial pneumonia with increased tendency to develop abscess, cavitation and empyema in camels **(Zubair *et al*, 2004; Kane *et al*, 2005; Abubakar *et al,* 2010)**. Enterotoxins are chromosomally or plasmid encoded. Enterotoxins are frequently cytotoxic and kill cells by altering the apical membrane permeability of the mucosal (epithelial) cells of the intestinal wall. They are mostly pore-forming toxins (mostly chloride pores), that assemble to form pores in cell membranes. This causes the cells to die **(eMedicine, 2008)**.Bacterial toxins were detected by its action on mammalian cell lines, causing changes in cell shape. These changes are defined as cytopathic effects and some of those denominated cytotoxin lead to death of cultured cells. *Klebsiella* spp. damages intestinal epithelium through producing cytotoxin tilivalline.Thistoxin plays a rolein the pathogenesis of bacteria on the host cells **(Thelestam and Florin, 1994 and Darby *et al.,* 2014)**. *Klebsiella* species has multifactorial virulence mechanism, which includes capsular polysaccharides (CPS), lipopolysaccharide (LPS), iron-scavenging systems (siderophores), adhesins and hypermucoviscosity **(Podschun and Ullmann, 1998).** Capsular polysaccharide biosynthesis is mainly governed by the flanking regions of *mag*A (mucoviscosity-associated gene A) which is strictly associated with K1 capsular serotype **(Struve *et al.,* 2004; Fang *et al.,* 2005**) and extracapsular polysaccharide synthesis is associated with plasmid mediated *rmp*A (regulator of the mucoid phenotype A) gene **(Nassif *et al.,* 1989)**. Both genes have been reported to increase the mucoviscosity and virulence, resulting in severe septicaemia and death **(Yoshida *et al*., 2000; Chuang *et al.,* 2006)**.

The *tra*T is one of a group of outer membrane protein (OMP) genes which encoded as an important virulence factors that are linked with human serum resistance **(Kuo *et al.,* 2017).** Adhesins governed by *fim*H which is an adhesive subunit of type 1 fimbriae. *Klebsiella pneumoniae* is a frequent cause of sepsis, urinary tract infection (UTI), and liver abscess. Type 1 fimbriae have been shown to be critical for the ability of *K. pneumoniae* to cause UTI. *fimH* gene is found in 90% of strains from various environmental and clinical sources **(Rosen *et al.,* 2008*)*.** Iron is one of the essential macro-nutrients for most bacterial species as it plays an important role in the electron transport chain and for various other enzymes as cofactor to grow successfully in host tissues .Siderophore; aerobactin, a hydroxamate siderophore whose receptor is encoded by *iut*A **(Bachman *et al.,* 2011)**.This study aimed to determine the prevalence of *Klebsiella* spp. in camel carcasses, and determine the virulence of *Klebsiella* isolates by phenotypic and molecular assay.

MATERIAL AND METHODS

Samples:

Six hundred samples were collected from lung, liver, kidney and spleen of 150 apparently healthy camels from slaughterhouses of El-kaliobia and El-Monofia governorate during years (2017-2018) .The samples were transported to the laboratory as quick as possible.

Isolation of *Klebsiella* species:

The collected samples were incubated in peptone broth for 24 hours at 37˚C aerobically and cultivated at solid plate media MacConkey's Bile Salt agar media (Oxoid CM7), Eosin Methylene Blue agar (Oxoid) incubated at 37˚C for 24 hours .the suspected mucoid colonies representing typical appearance. Colonies were picked and propagated in nutrient agar slope for further identification **(Koneman, 2006; Gerald, 2012)**.

Identification of *Klebsiella* species:

The purified colonies were subjected to Morphological examination .Films from the pure colonies were stained by Grams stain and examined microscopically .the colonies that consisted of organisms to be gram negative ,straight rods with parallel or bulging sides, were further subjected to biochemical identification **(Barbara ,1994).**

Congo red (CR) Binding test:

The isolated strains were tested for their growth status on congo red medium .the reaction was seen after 18-24 - 48 and 72 hr of incubation at 37˚C and was left at room temperature for an additional 2 days the congo red positive (CR+) were appeared as bright or orange red colonies .Different intensities in the dye uptake were (+) and (++).Congo red negative (CR-) isolates did not bind the dye and appeared as white colonies **(Berkhoff and Vinal, 1986)**.

Haemagglutination test (HA):

Different blood samples were taken from various animals species (cattle, sheep, horse and human group O). 1ml of 3.8% citric acid in distilled water was added to 9.0 ml the blood .Blood was diluted 1:4 with phosphate buffered saline PH 7.2 to test for (HA) and 1:4 with 1%D-mannose to test for mannose resistance haemagglutination (MRHA).Bacterial cells from *klebsiella* isolates were obtained from cultures grown for 18 hrs at 37 ˚c on colonization factor agar (CFA)plates .Growth was picked up and mixed with a drop of the different blood species of blood on a microscopic slide at room temperature and observed for 1-2 min . Haemagglutination was observed by the naked eye supported by the aid of a hand lens .Haemagglutination was recorded as mannose resistant if occurred with and without D-mannose and as mannose sensitive if HA was inhibited in the presence of D- mannose **(Evans *et al*., 1979)**.

Suckling mouse assay:

Klebsiella strains tested for production of heat stable enterotoxin by suckling mouse assay according to **Giannella, 1976**. Strains were grown in casamino acid yeast extract and 0 - 1 ml was injected intragastrically in mice 2 - 4 days old. After three hours the mice were killed by decapitation and the ratio from gut weight divided by body weight was determined. Values greater than 0 - 0.83 were considered positive.

Assay of cytotoxicity:

The procedure of **Raja *et al*., 2010** was adopted. Cell Free Culture Supernatants of the 44 *Klebsiella* isolates were prepared. Each bacterial strain was grown in Brain Heart Infusion broth. All of the culture supernatants were adjusted to pH 7.3 -7.5. One hundred microliters of 5×10^{4} tissue culture cells per milliliter in Eagle's minimal essential medium containing kanamycin and 10% fetal calf serum was added to the wells of 96-well microtiter plates and incubated in air containing 5% CO2 at 37C° for 2 days. Aliquots of 25 ml of the cell-free culture supernatant of *Klebsiella* diluted in a two-fold series with Dulbecco's phosphate-buffered saline (pH 7.4)

were then added to 100 ml of the monolayer cultures in a well, and the microtiter plates were incubated at 37C^0 in CO2 incubator for 48 hours. The results of the cytotoxicity assay was expressed as a reciprocal of the highest final dilution of culture supernatant that caused rounding of cultured cells in the wells**.**

Detection of virulence gene of *k.pneumoniae and k. Oxyocae* by PCR;DNA were extracted according to QIAamp DNA mini kit instructions according to **(Soumet *et al* ., 1999 and yang *et al*., 2014)**, preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara) Code No.RR310A kit. Oligonucleotide primers used in cPCR were showed in (Table 1).Amplified PCR product was electrophoresed in 1.5% agar by agarose gel electrophoreses according to **Sambourk *et al.,* 2001** and visualized by U.V transilliuminator.

RESULT

Bacteriological examination of internal organs of camels revealed isolation of *klebsiella* strains with an overall incidence 29.3% as shown in (Table 2).The *klebsiella* isolates recovered from internal organs of apparently healthy camel with incidence of 10.6%, 7.3%, 7.3% and 4% from lung, liver, kidney and spleen respectively. The biochemical identification of the 44recovered isolates revealed12 K. pneumoniae*, 26 K.oxytoca* and 6 *K.rhinoscleromatis.* Results of Congo red binding activity of 44 *Klebsiella* species isolated from apparently healthy camels are demonstrated according to (Table 5). All isolates gave positive result with different degree in red color, 28 (63.6%) gave deep red color and 16 strains (36.3%) gave pale orange, while the haemagglutination pattern of *Klebsiella* species isolated from apparently healthy camels against erythrocytes of different species via: Cattle, sheep, horse and human (group O) are illustrated in (Table 6). Result of mannose resistant haemagglutination pattern of *K.pneumoniae* isolates against erythrocytes of cattle, sheep ,horse and human (group O) were 7,8,5 and 6 respectively ,while it was 3,2,4,4 for *k. rhinoscleromatis* and 18,15,18,14 for *k. oxytoca*. Eight *K. pnemoniae* 15 *K. oxytocae* and 2 *K .rhinoscleromatis* were positive for production of enterotoxin .the ratio high or equal to 0.085 were positive while lower than 0.085 Considered negative as shown in (Table 7). 32 isolate out of 44 with an incidence of 72.7 % were cytotoxic to vero cells that induce severe vaculation and detachment. Cytopathic effect was recorded in 10, 18, and 4 for *k.pneumonia*e, *k. oxytoca*, *and k. rhinoscleromatis* respectively, the cells having abnormal appearance. *Klebsiella pneumonia* isolates were highly cytotoxic to vero cell with an incidence of 83.3%, *Klebsiella* oxytoca and *Kebsiella rhinoscleromtis* were cytotoxic to vero cells with

an incidence of 69.2% and 66.6% respectively (Table 8). 24 *Klebsiella* strains (12 *K.pneumoniae* and 12 *K. oxytoca*) examined for presence of virulence genes *fim*H ,*tra*T ,*mag* A and *iut*A .It was noticed that all 24 strains were positive to *fim*H ,*tra*T that are amplified 508 and 300 bp respectively (Table 9), Fig. (1, 4). 23out of 24 strains harbor *iut*A (Table 9), Fig. (3), while all 24 isolates don't harbor *mag* A (Table 9), Fig. (2)

DISCUSSION

Africa has the biggest population of camels in comparison to other continents **(Hamza *et al.,* 2017)**.Camels migrate between the open boundaries of Sudan and Egypt either for grazing or for slaughtering. Bad hygiene and stress is often related to pulmonary diseases in camels **(Wareth *et al.,* 2014).** However few literatures were published on the incidence of *Klebsiella* in camels. *Klebsiella* species were recovered from internal organs of 150 camels carcasses in slaughter houses with an overall incidence 29.3% (Table 2).*Klebsiella* could be isolated from lung, liver, kidney and spleen .The highest incidence of isolation was in lung 10.6% followed by liver and kidney 7.3% for each and finally spleen 4% (Table 3).This finding is higher than that reported by **Hamza *et al.,* (2017)** who recovered *Klebsiella* species from internal lesions of lung, liver and heart with a prevalence rate 4%.while it was agree to large extent with **Al-Tarazi *et al* (2001)** who recovered *Klebsiella* species from lung with an incidence 10.2%. .*Klebsiella* could be recovered from liver of camels with percentage rate 3.3% (**Aljameel *et al*., 2014).**Biochemical identification revealed that 12 isolates were *K.pneumoniae* (5.2%),26 *K. oxytoca* (11.4%) and 6 *K. Rhinoscleromatis* (2.6%). *K.pneumoniae* was recovered from camels with a percentage rate 20.7% (**Mansour *et al*., 2014).** *K.pneumoniae* was recovered from healthy camels (37%) , diarrheioic (45%) convalescent (3%) and dead camel calves(15%) aged from birth to12 weeks **(younnan *et al* ., 2013). and Dia *et al* .,(2000)** isolated eight *K.pneumoniae* out of 29 cases calf diarrhea. It was noticed that the highest isolation rate of *K.pneumoniae* (4%) from lung while it was from liver for *K. oxytoca* (6%), this could probably indicated a concurrent extra intestinal infection (Table 4). **Al-Tarazi *et al* ,(2001)** declare that *Klebsiella* species recovered from interstitial pneumonia with an incidence 14.6% .and *Klebsiella ozonae* the most frequent among *Klebsiella* species identified as; 5 *K. ozonae*, 3 *K.pneumoniae*,2*K.oxytoca*.1 *K.rhinoscleromatis*. In the current study all *K. pneumonia* were indole negative and *K. Oxytoca* were indole positive our result agreed with **Hansen *et al*.,(2004)** who reported that applying 18 different biochemical test on 242 strains showed that

K.pneumoniae were indole negative while *K. oxytoca* were indole positive, while **Sharma *et al* .,2017** compare between *K.pneumoniae* isolates recovered from nasal discharge of pneumonic (47) and healthy (18) camels, all *K.pneumoniae* isolates showed typical inherent phenotypic pattern except six isolates from pneumonic camels were found ability to produce indole. All of the *Klebsiella* isolates gave positive result with different degree in red colour, 28 (63.6%) gave deep red colour and 16 strain (36.3%) gave pale orange (Table 6). **Jagnow and Clegg, (2003); Schroll, Barken *et al,* (2010) and Huertas, Zarate *et al,* (2014)** said that, the major biofilm components were identified by spotting the bacterial culture on Congo red agar plates. The *Klebsiella* species resulted in an RDAR (red, dry, and rough) colony. Mannose resistant haemagglutination pattern of *K. pneumonia* isolates against erythrocytes of cattle, sheep, horse and human (group O) were 7, 8, 5, and 6 respectively. It was 3,2,4,4 for *K .rhinoscleromatis* and 18,15,18,14 for *K. oxytoca* respectively (Table 7). Most *K.pneumoniae* express type 1and type 3 fimbriae which are characterized by mediation of mannose sensitive agglutination and agglutination of tannic acid treated red blood cells **(Ong *et al*., 2010, Stahlhut *et al.,* 2012).** Eight, 15 and 2 *K.pneumoniae, K.oxytocae and K.rhinoscleromatis* were positive for production of enterotoxin respectively (Table 8). Heat-labile and heat-stable enterotoxin leads to increased chloride ion permeability of the apical membrane of intestinal mucosal cells. These membrane pores are activated either by increased cAMP or by increased calcium ion concentration intracellular. The pore formation has a direct effect on the osmolality of the luminal contents of the intestines. Increased chloride permeability leads to leakage into the lumen followed by sodium and water movement **(Dworkin *et al.,* 2006 and Hornby, 2015).**The cytotoxic activity was illustrated in (Table 9). It is obvious that 32 isolates (72.7 %) were cytotoxic to vero cells induce severe vaculation and detachment .Cytotoxin is one of the important pathogenic factors, which plays a role in the virulence of this bacterium. 100% cytotoxicity and lethality were observed with *K.pneumoniae* K1 and K2**(Osman *et al*., 2014).** *Klebsiella* strains exhibit different virulence factors such as capsular polysaccharides (CPS), lipopolysaccharide(LPS), iron-scavenging systems (siderophores), adhesins , hypermucoviscosity and outer membrane lipoprotein (**Sharma *et al* .,2015)**. All 24 examined isolates harbor *fim*H gene (table 10, fig 6) *fim*H gene was detected in 80 % of *K. pneumoniae* **(Wasfi *et al.,* 2016).** The *fimH* gene give the hypermucoid properties of *Klebsiella* causing high resistance to phagocytosis, reduced serum sensitive and were more virulent in animal studies in comparison with non-mucoid

isolates. The invasion *mag*A gene (mucoviscosity-associated gene A) could not be detected (Table 10 fig 4). 14 strains of 177 isolated strains harbor *mag*A gene as it restricted to K1 isolates **(Hartmen *et al*., 2009)** *mag*A gene more often found in liver invasive strains (98%) as compared to non-liver-invasive strains (29%) **(Wiskur, Hunt and Callegan 2008**). *Twenty three strains out of 24* harbor *iut*A gene (Table 10) Fig.(5) *iut*A gene was detected in all 15 strains **(Compain *et al* .,2014)**. Iron is present as complex to carries molecules like transferrin, lactoferrin and heme, bacteria must be able to obtain iron from these host transport proteins. At this lower iron condition, bacteria produce siderophore, low molecular weight iron chelators to solubilize and utilize iron. In *Klebsiella*, three siderophore systems are present: enterobactin, aerobactin, and yersiniabactin (**Lawlor, O' Connor, Miller 2007)**.The *tra*T locus includes the pillin gene and regulatory genes, which together form pili on the cell surface, polymeric proteins that can attach themselves to the surface of the F bacteria and initiate conjugation, the existence of *tra*T is on a plasmid **(Grohmann, Muth and Espinosa, 2003**).The *traT* gene was detected in all strains (100%) (Table 9) *tra*T gene was detected in (78.5%) of *K. pneumoniae* **(Wasfi *et al.,* 2016).** On the other hand **Kuo *et al.,* (2017)** could detect *tra*T gene in 11.3% of *K. pneumonia* isolates. In conclusion presence of *klebsiella* in apparently healthy camels prompted us to identify these pathogenic agents that are associated with pneumonia and predispose to other infections in this neglected animal species. In this study we review factors that may mediate virulence (invasion, adhesion, sidrophores and endotoxin) *as Klebsiella* infections contribute pathogenicity to humans.

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Table (1): Oligonucleotide primers sequences: Source: Metabion (Germany).

Target gene	Primers sequences (5/-3/)	Amplified segment (bp)	Reference
***iut*A**	**GGCTGGACATGGGAACTGG**	**300**	**Yaguchi *et al.*, 2007**
	CGTCGGGAACGGGTAGAATCG		
***fim*H**	**TGCAGAACGGATAAGCCGTGG**	**508**	**Ghanbarpour and Salehi, 2010**
	GCAGTCACCTGCCCTCCGGTA		
***tra*T**	**GATGGCTGAACCGTGGTTATG**	**307**	**Kaipainen *et al.*, 2002**
	CACACGGGTCTGGTATTTATGC		
***mag*A**	**GGTGCTCTTTACATCATTGC**	**1282**	**Yeh *et al.*, 2007**
	GCAATGGCCATTTGCGTTAG		

Table (2): Prevalence of *klebsiella* species recovered from camels.

Number of Camels	n=sample	n=positive sample	Ratio
150	**600**	**44**	**29.3% ***

***the percentage is calculated according to total no.of camels.**

Table (3): The incidence of *klebsiella* species isolated from internal organs of apparently healthy camels.

Organs(150)	n=positive sample	Percentage *
Lung	16	10.6 %
Liver	11	7.3 %
Kidney	11	7.3 %
Spleen	6	4%

***The percentage is calculated according to total no. of organs.**

Table (4): Prevalence of different *klebsiella* species in examined samples.

Samples(no.150)	*K.pneumoniae*		*K.oxytoca*		*K.rhinoscleromatis*	
	n=	%*	n=	%*	n=	%*
Lung	6	4	6	4	4	2.6
Liver	2	1.3	9	6	-	0
Kidney	3	2	6	4	2	1.3
Spleen	1	0.6	5	3.3	-	0
Total	12	27.3	26	59.1	6	13.6

***The percentage is calculated according to total no. of organs.**

***The percentage is calculated according to total no. of isolates (44).**

Table (5): Degree of Congo red binding activity of *Klebsiella* species isolated from apparently healthy camels.

Klebsiella species	n=examined isolates	n=CR++	n=CR+
K.pneumoniae	12	8	4
K. rhinoscleromatis	6	4	2
K. oxytoca	26	16	10
Total	44	28	16

CR++ =Congo red strong positive CR+ = Congo red positive

Table (6): Haemagglutination of different erythrocytes by *Klebsiella* isolates recovered from camels.

Species of RBCs	Type of haem agglutination	*k. pneumonia (n=12)*		*K. .rhinosclero matis (n=6)*		*k. oxytoca (n=26)*		*Total (n=44)*	
		n=	%	n=	%	n=	%	n=	%
Cattle	**MRHA**	**7**	**58.3**	**3**	**50**	**18**	**69.2**	**28**	**63.6**
	MSHA	**2**	**16.6**	**2**	**33.3**	**4**	**15.3**	**8**	**18.1**
	HA+	**9**	**74.9**	**5**	**83.3**	**23**	**84.5**	**37**	**84**
Sheep	**MRHA**	**8**	**66.6**	**2**	**33.3**	**15**	**57.6**	**25**	**56.8**
	MSHA	**3**	**25**	**1**	**16.6**	**6**	**23**	**10**	**22.7**
	HA+	**11**	**91.6**	**3**	**49.9**	**21**	**80.6**	**35**	**79.5**
Horse	**MRHA**	**5**	**41.6**	**4**	**66.6**	**18**	**69.2**	**27**	**61.3**
	MSHA	**1**	**8.3**	**0**	**0**	**2**	**7.6**	**3**	**6.8**
	HA+	**6**	**49.9**	**4**	**66.6**	**20**	**76.8**	**30**	**68.1**
Human(o)	**MRHA**	**6**	**50**	**4**	**66.6**	**14**	**53.8**	**24**	**54.5**
	MSHA	**3**	**25**	**1**	**16.6**	**5**	**19.2**	**9**	**20.4**
	HA+	**9**	**75**	**5**	**83.2**	**19**	**73**	**33**	**75**

MRHA:mannoseresistanthaemagglutinationMSHA:mannosesensitive haemagglutination

Table (7): Showing Enterotoxigenic activity of *Klebsiella* species.

Klebsiella species	n=examined isolates	GW/BW ratio
K. pneumoniae ***K. rhinoscleromatis*** ***K. oxytocae***	**12** **6** **26**	**8≥0.085** **4<0.085** **2≥0.085** **4<0.085** **15≥0.085** **11<0.085**

Table (8): showing Cytotoxicity of *klebsiella* species isolated from Camel.

Klebsiella species	n= examined isolates	n=Positive	%
K. pneumonia	**12**	**10**	**83.3**
K. oxytoca	**26**	**18**	**69.2**
K. rhinoscleromatis	**6**	**4**	**66.6**
Total	**44**	**32**	**72.7**

Table (9): Comparison of virulence genes in *K. pneumonia* and *K. oxytoca*. By PCR.

k.pneumoniae	*iut*A	*mag*A	*Tra*T	*fim*H	*k.oxytoca*	*iut*A	*mag*A	*tra*T	*fim*H
1	+	-	+	+	13	+	-	+	+
2	+	-	+	+	14	+	-	+	+
3	+	-	+	+	15	+	-	+	+
4	+	-	+	+	16	+	-	+	+
5	+	-	+	+	17	+	-	+	+
6	+	-	+	+	18	+	-	+	+
7	+	-	+	+	19	-	-	+	+
8	+	-	+	+	20	+	-	+	+
9	+	-	+	+	21	+	-	+	+
10	+	-	+	+	22	+	-	+	+
11	+	-	+	+	23	+	-	+	+
12	+	-	+	+	24	+	-	+	+

24 23 22 21 20 19 18 17 16 15 14 13 m 12 11 10 9 8 7 6 5 4 3 2 1 m c+ c-

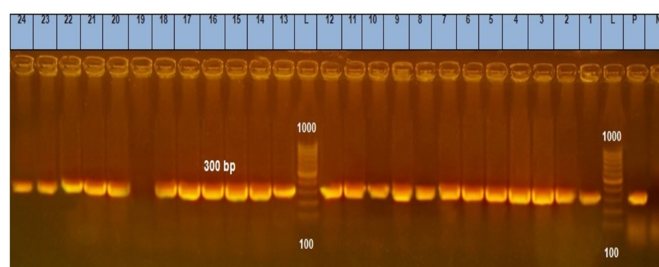

Fig. (1): Agarose gel electrophoresis showing pcr amplification at 300 bp for *iut*A gene of *K. pneumoniae* and *K. oxytoca* respectively.

Lane (L): 100 bp DNA marker (Qiagen), lane (pos) control *K. pneumoniae* ATCC®13883 and lane (Neg) negative control *E.Coli* ATCC®8739. lane 1,2,3,4,5,6,7,8,9,10,11,12 positive samples *K. pneumoniae* , lane 13-,14,15,16 ,17 ,18 ,20,21,22,23,24 positive for *k. oxytoca* and 19 negative for *K. oxytoca* .

24 23 22 2120 191817 16 15 1413 12 11 10 9 8 7 6 5 4 3 2 1 c- c+ m

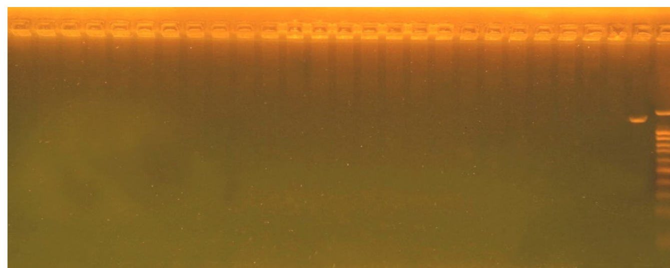

Fig. (2): Agarose gel electrophoresis showing pcr amplification at 1282 bp for *mag*A gene of *K.pneumoniae* and *K. oxytoca* respectively.

Lane (L): 100-1500 bp Gel Pilot 100 bp plus ladder (Qiagen), lane (pos) control *K.pneumoniae* ATCC®13883 and lane (Neg) negative control *E. Coli* ATCC®8739. Lane 1,2,3,4,5,6,7,8,9,10,11,12 negative sample *K.pneumoniae* , lane.
13,14,15,16,17,18,19,20,21,22,23,24 negative sample *k. oxytoca* .

24 23 22 21 20 19 18 17 16 15 14 13c+ m 12 10 9 8 7 6 5 4 3 2 1 c-

307 bp

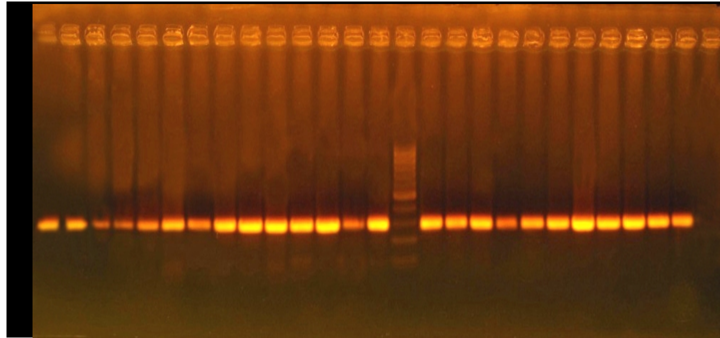

Fig. (3): Agarose gel electrophoresis showing amplification at 307 bp fragment of *tra*T gene of *K.pneumoniae* and *K. oxytoca* respectively.

Lane (L): (100 bp DNA marker (Qiagen). lane (pos) control *K.pneumoniae* ATCC®13883 and lane (Neg) negative control *E. Coli* ATCC®8739 .lane 1,2,3,4,5,6,7,8,9,10,11,12 positive sample *K.pneumoniae* , lane 13, 14 ,15, 16, 17, 18, 19, 20, 21, 22,23 ,24 positive sample *k .oxytoca*) .

24 23 22 2120 19 18 17 16 15 14 13 c+ m 12 11 10 9 8 7 6 5 4 3 2 1 c-

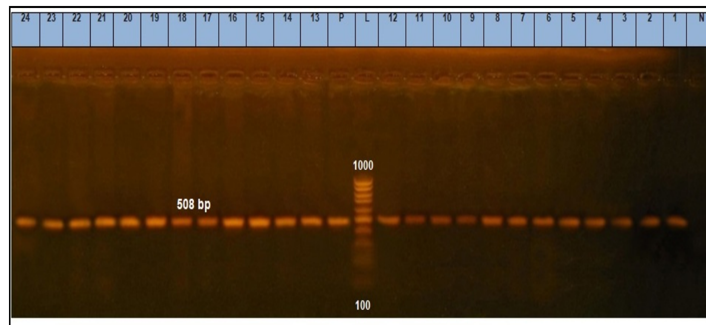

Fig. (4): Agarose gel electrophoresis showing amplification at 508 bp fragment for *fim* H gene of *K.pneumoniae* and *K. oxytoca* respectively.

Lane (L):100 bp DNA marker (Qiagen), lane (pos) control *K.pneumoniae* ATCC®13883 and lane (Neg) negative control *E. Coli* ATCC®8739.lane 1,2,3,4,5,6,7,8,9,10,11,12 positive samples *K.pneumoniae* , lane 13-,14,15,16 ,17 ,18 ,20,21,22,23,24 positive for *k. oxytoca* and 19 negative for *K. oxytoca* .