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Research Article

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Isolation of *Klebsiella pneumoniae* from urine of human and cattle in Baghdad city with histopathological study experimentally in mice

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Abstract

This study encompass four accessories: part one was proceeded to isolate *Klebsiella pneumoniae* from human and animals, urine specimens were collected from 311 diseased human suffering urinary tract infection (UTI), various ages, in both sexes from Mohammed Bakir Al –Hakeem hospital and Al-Shulla city /Baghdad, and urine samples from150 cattle of Al-shulla –slaughter house, the specimens collected were (100) samples from Al-dehana city shulla and (3) samples were collected by Veterinary hospital/ Aden square teem \ during immunization program of farm animal and Al-Qahera City (47) samples were collected from privet slaughter house.

The results presentation that *Klebsiella pneumoniae* were isolated in (6) out of (311) human patients with UTI, urine samples were 1.9% from isolates that confirmed *Klebsiella pneumoniae* . The number of *Klebsiella pneumoniae* isolates from cattle urine sample were (22) out of (150) (14.9%) from isolates were *Klebsiella pneumoniae* .

The histopathological study was included estimating the infectious dose (ID) of *Klebsiella pneumoniae* in mice and experimental induction of infective dose in mice.

Twenty five mice (males and females), aged 6- 8 weeks and weighing 25-30 gram, had negative urine bacteriological culture of *k Klebsiella pneumoniae* were used. The mice were randomly divided into two groups: Infected group **(**25 mice), each animal was infected by injection 0.2 I.P containing (7.5×10^4 C.F.U.) of the prepared infectious dose of *K. pneumoniae* , and control group (10 mice) were injection 0.2 PBS dose, then looking for clinical signs daily for 2 weeks.

The animals were killed after (24, 48, and 72) hours, 1 week and 2weeks

Finally **s**pecimens from internal organs including: liver, and intestine, heart, spleen, kidney and lung after immolate mice at (24, 48, 72) hours, 1 week and 2weeks from infected and control groups were used for histopathological examination.

Histopathological changes after (24, 48, 72) hours and 1 week of infection with *Klebsiella pneumoniae* were the most extremity from the group after 2 weeks of infection especially in kidney that showed exhibited desquamation of epithelial cells linning the degenerative cells of renal tubules and infiltration of inflammatory cells around glomeruli and dilatation of glomerular space of the kidney, congestion of blood vessels and marked inflammatory cells aggregation around the portal areas and in the interstitial tissue of the liver and hyperplasia of endothelial cells of the central artery with hyperplasia periarterial area of spleen. Intestine shows few inflammatory cells infiltration in the submucosa and lamina properia of intestinal mucosa, desquamation of some epithelial cells and congestion of blood vessels.

Increased thickness of intra alveolar septa of the lung due to congested capillary blood vessels and inflammatory cells infiltration in lung and inflammatory cells aggregation between cardiac muscle fiber in the heart.

Keywords: UTI, *Klebsiella pneumoniae* , urine samples, Histopathological study.

Introduction

Klebsiella pneumoniae is considered as a saprophyte in humans and other mammals, colonizing the gastrointestinal tract, skin, and nasopharynx is a member of the *Klebsiella* genus of Enterobacteriaceae. *K. pneumoniae* is found in the environment and as a harmless commensally, but is also a frequent nosocomial pathogen (causing urinary, respiratory and blood infections) (Tzouvelekis *et al* .,2012),. The emergence of *K.pneumoniae* as a nosocomial pathogen in the US and Europe may be due in part to the acquisition of antibiotic resistance markers providing a selective advantage in hospital settings, and it is responsible for 6-17% of urinary tract infection (UTI's), 7-14% of pneumonia, 4-15% of septicemia, 2-4% of wound infections, 4-17 nosocomial infections in intensive care units, and 3-20% of all neonatal septicemia cases (Fodah *et al*.,2014).

Factors that are implicated in the virulence of *K. pneumoniae* strains include the capsular serotype, lipopolysaccharide, iron scavenging systems, and fimbrial and non-fimbrial adhesions. The abundant polysaccharidic capsule that typically surrounds *K. pneumoniae* that isolated from urine with 41% as isolation. So the aims of this study are isolation of *Klebsiella pneumoniae* from human patients and from cows that suffering from urinary tract infection (U TI) and experimental Infection in mice with study of histopathological changes (Struve and Krogfelt, 2004).

Materials and Methods

Collection of urine samples

1. Human samples

Three hundred and eleven urine samples from patients suffered from urinary tract infection were collected during the study period from (December / 2015 – April / 2016). The patients were attended from Department of microbiology laboratory of Mohammed Bakir Al –Hakeem hospital e /Al-Shulla city /Baghdad and from patients dormant in hospitals.

2. Animal samples

One hundred and fifty urine samples were collected randomly from cattle with apparently healthy and non healthy, of different age and sex, and were collected during the study period from (october /2015 December / 2015), in the following geographical areas:

1-Al- Shula –slaughter house: The samples were collected was (100) sample
2-Al-dehna city Shula: (3) samples were collected by Veterinary hospital/ Aden square teem \ during immunization program of farm animal.
3- Al-Qahera City: forty seven samples were collected.

Isolation of *Klebsiella pneumoniae*

All sample were immediately streaked onto MacConkey, and incubated at 37˚C for 24hours., and streaked again on Brilliant Green Agar and Xylose lysine De ox ycholate Agar (xld) and incubated at 37˚C for 24hr., and streaked again on Hi chrom uti agar this medium is selective which used for the identification of *K. pneumoniae* gram stain (Morello *et al.*, 2006). Then confirm the isolates by using biochemical tests and ApI 20 E system.

Experimental animals

60 healthy mice, (male and female), aging (4-6) weeks old, weight from (25-30gm) were randomly divided into six group, the mice were reared in separate cages in the animal house of zoonotic research unit in the College of Veterinary Medicine / University of Baghdad, the animal feed on, pellets and water.

Determination of the infected dose

Prepared the bacterial suspension for infected dose the method as Hsu *et al*., (2011), was carried out. The mice were infected with 0.2 ml of 7.5×10^4CFU/ml, and infectious dose in mice inject I/P.

Experimental infection in mice

Thirty five mice of both sexes were randomly divided into five groups treated as follows: First group were divided into five -subgroups five mice for each subgroup and, injection intra peritoneally with (0.2ml) bacterial suspension containing $7.5x10^4$ CFU/ml of *Klebsiella pneumoniae* pathogen. Animal were sacrificed after 24 hours ,48 hours,72 hours,1 week and 2week after infection .Then organ takes and study gross change ,then pathological study all organ fixed in 10% formalin . Second group ten mice were injection intra peritoneally with sterile normal saline and were served as control group.

Mice were killed after, 24, 48, 72hrs, 1 week, 2 week of bacterial injection for histopathological study.

Results

Klebsiella pneumoniae in human urine:

Urine samples that collected from patient human revealed that 6 out of 311 were positive for *Klebsiella pneumoniae* Table (1).

Table (1): Number and % of *Klebsiella pneumoniae* patient human urine.

Origin of samples	No. of samples	No. of *Klebsiella pneumoniae* isolates	%
patient human urine	311	6	1.92%

Klebsiella pneumoniae animals urine

The number of *Klebsiella pneumoniae* isolates in cattle urine samples were22 from 150 samples, (Table 2).

Table (2): Number and % of *Klebsiella pneumoniae* in animals urine samples (cattle).

Origin of samples	No. of samples	No. of *Klebsiella pneumoniae* isolates	%
Cattle urine samples	150	22	14.66%

Isolation, characterization and serotyping of *Klebsiella pneumoniae*

The collected specimens were streaked directly on MacConky agar incubated at 37ºC for 24 hours, the large, pink, mucoid colonies were selected and subcultured on another Brillant green agar and on X.L.D and blood agar to obtain discrete colonies by the following steps:

1. Cultural characteristics

Initially different morphological shape and color of *Klebsiella pneumoniae* colonies was appeared on media, pink color on MacConkey agar, very mucoid while on Brillant green yellow to green the colonies appeared and yellow colour on X.L.D media, Gram negative rod, mucoid non spore forming under light microscope suspected as *Klebsiella pneumoniae*. (Figure, 1).

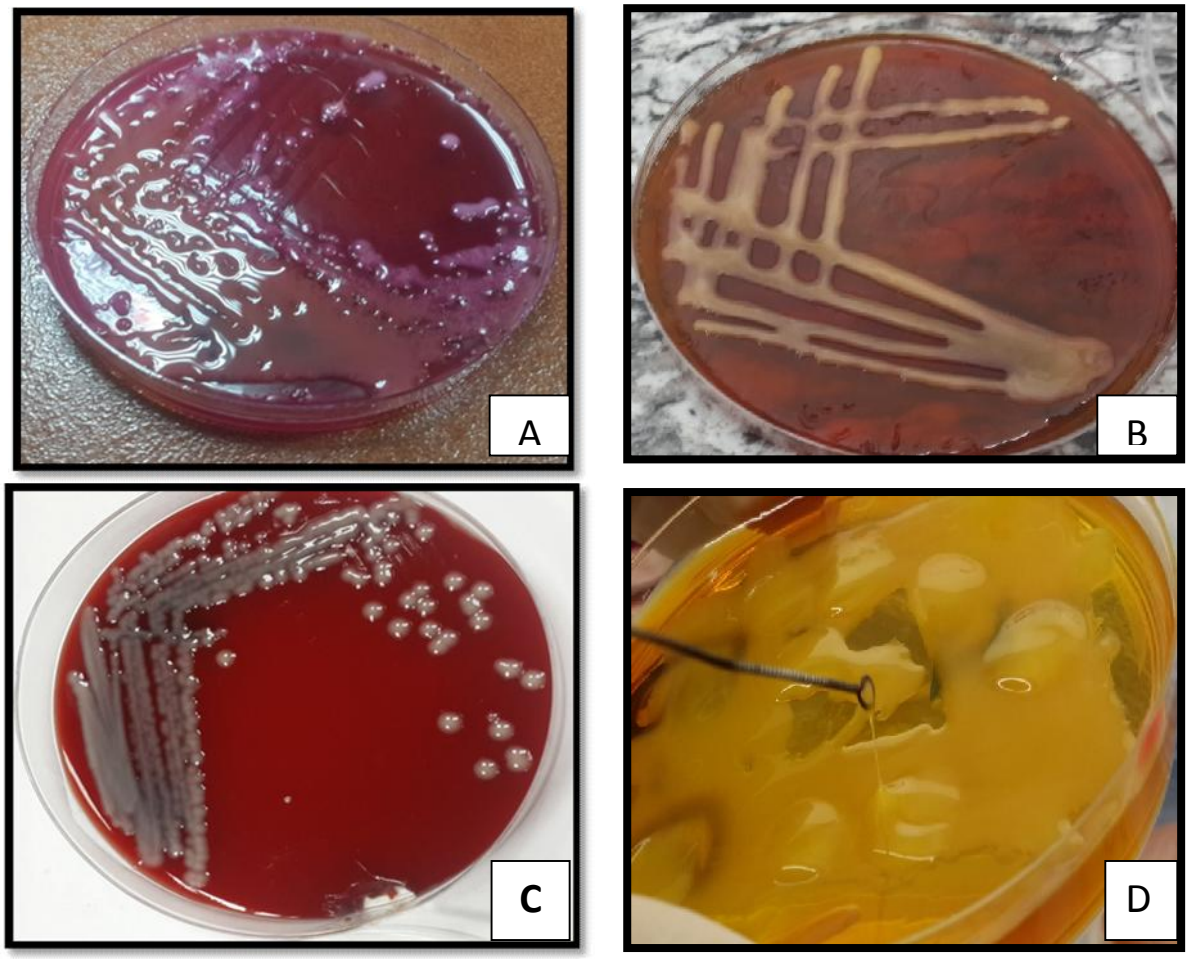


Figure (1): **Colonies of *Klebsiella pneumoniae* (A) on MacConkey (B) XLD agar (C) Blood agar (D) Briliantgreen agar**

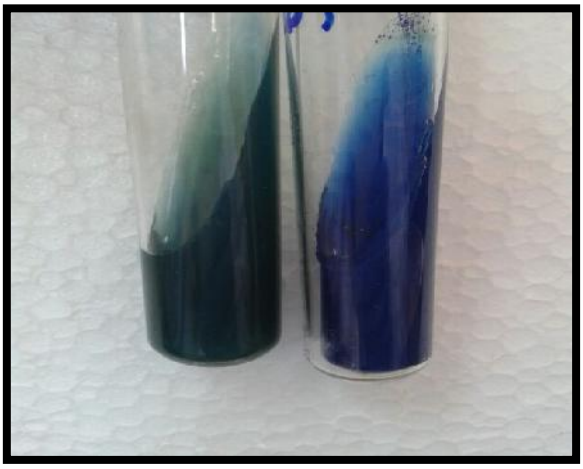
2. Biochemical identification

The biochemical tests of the isolated bacteria gave different results, the isolates gave positive results for Cimmon citrate & Urease. tests, VP while indole, MR and motility tests gave negative results, Triple sugar iron test showed Yellow/Yellow with gas production ,urea's positive ,change from orange to pink color as shown in (Table 1; Figure 2).

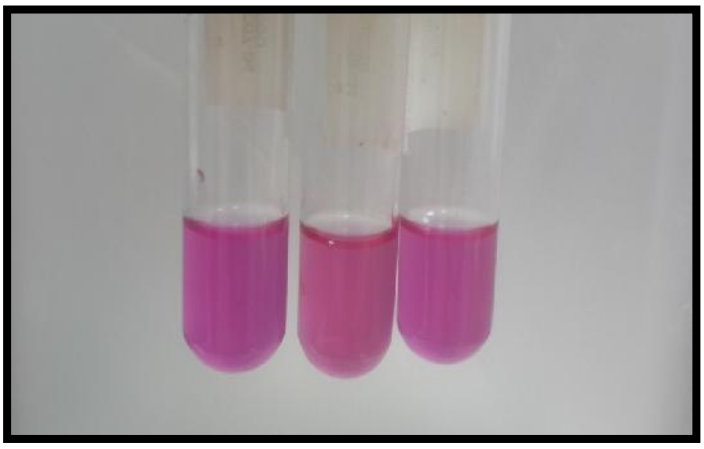
Table (1): The results of some biochemical test of *Klebsiella pneumoniae*.

Biochemical test	Result
Simmon citrate test	(+) ve
urease test	(+) ve
Methyl Red test.	(-) ve
Triple sugar iron	Yellow/Yellow with gas production
Indol test	(-) ve
Motility test	(-) ve

A- Triple sugar iron (+)



B- Simmon citrate test (+)



C-Urea's test (+)

Figure (2): showed Biochemical test of *Klebsiella pneumoniae*

Diagnosis by using API 20E system

Twenty two animal isolate from cattle urine of total (150) sample doing Api 20e , also(6) human isolate from patient human urine of total (311) sample . Our results show that all the isolates from human and cattle urine conformable to Api20e leaflet and the results are *Klebsiella pneumoniae*, and shown *Klebsiella pneumoniae* sup spp. *pneumoniae* as in (fig.3)

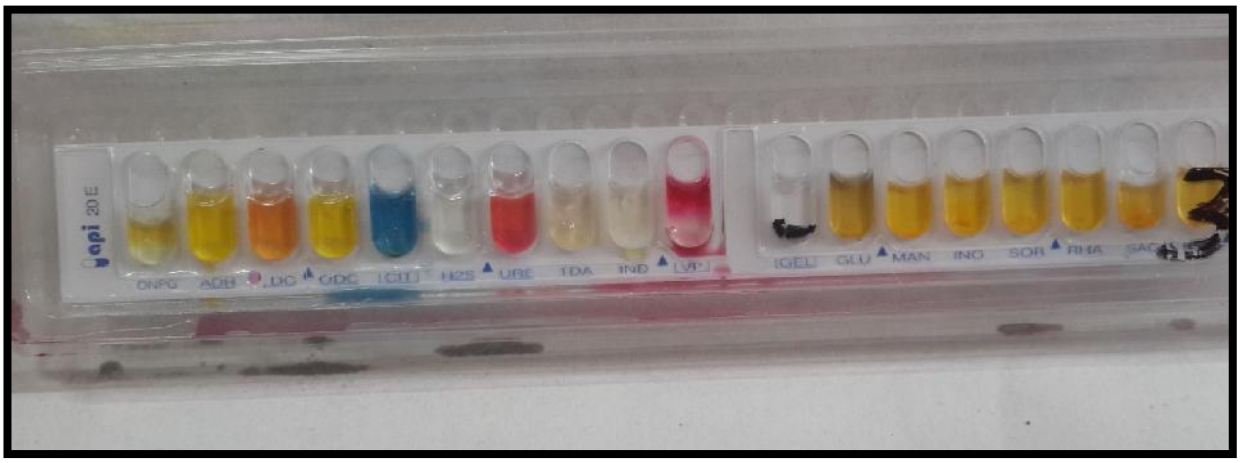


Figure (3) showed the positive (+ve) results of *Klebsiella pneumoniae* on Api 20E

Histopathological Study

The pathological examination of infection with *Klebsiella pneumoniae* for all groups were the same lesions to all internal organs that collected from infected animals but the infection was more intensive after 48, 72 hours of infection that exhibited congestion of blood vessels and infiltration of polymorphonuclear cells and kupffer cells in the sinuses and interstitial tissue (Fig.4). Histopathological section of the spleen showed hyperplasia of endothelial cells of the central artery and proliferation of megacarocyte (Fig.5), congested of blood vessels and inflammatory cells infiltration in the lung (Fig. 6). In kidney congestion of blood vessels, hemorrhage, infiltration of inflammatory cells in the interstitial tissue and dilatation of glomerular space (Fig.7), inflammatory cells infiltration in the submucosa and lamina properia of intestinal mucosa in intestine (Fig. 8) and inflammatory cells aggregation in the lumen blood vessels and between cardiac muscle fiber in the heart (Fig. 9).

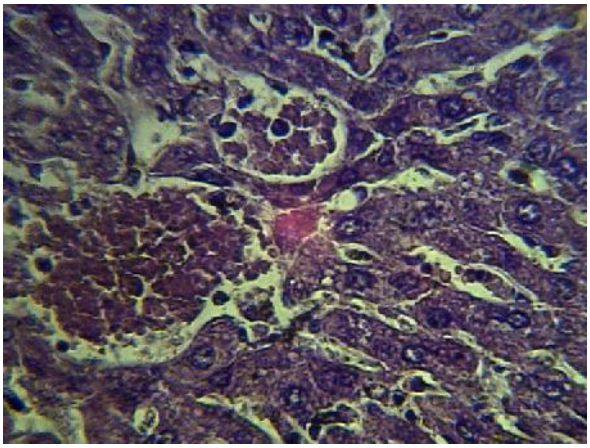


Fig 4: Histopathological section in liver of animal 48 hours of infection with *Klebsiella pneumoniae* shows congestion of blood vessels ( ) and infiltration of polymorphonuclear cells and kupffer cells in the sinuses and interstitial tissue (H&EX40).

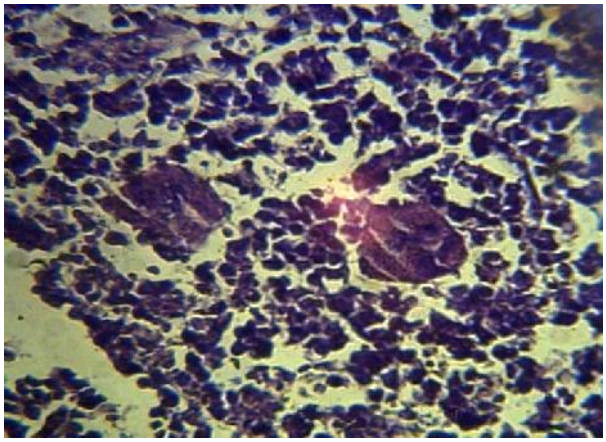


Figure 5: Histopathological section of the spleen of animal 48 hours of infection with *Klebsiella pneumoniae* showed hyperplasia of endothelial cells of the central artery and proliferation of megacarocyte (H&E X400).

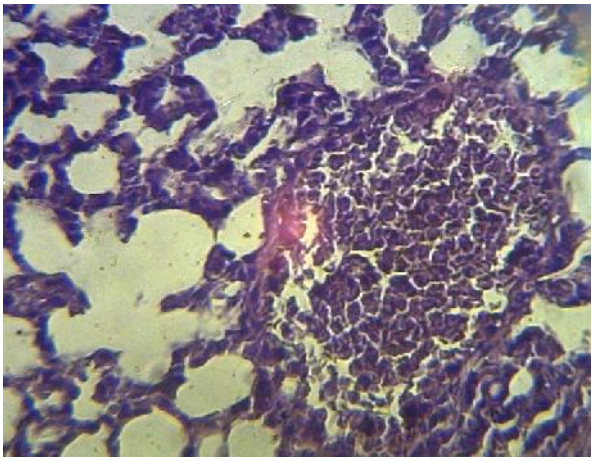


Fig 6:Histopathological section in lung of animal 48 hours of infection with *Klebsiella pneumoniae* showed congested of blood vessels and inflammatory cells infiltration (H&EX40). central artery and proliferation of megacarocyte (H&E X400).

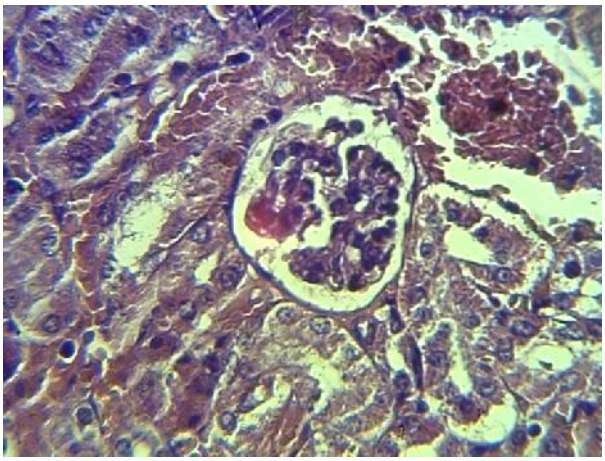


Fig 7: Histopathological section in kidney of animal 48 hours of infection with *Klebsiella pneumoniae* showed congestion of blood vessels, hemorrhage, infiltration of inflammatory cells in the interstitial tissue and dilatation of glomerular space (H&EX400).

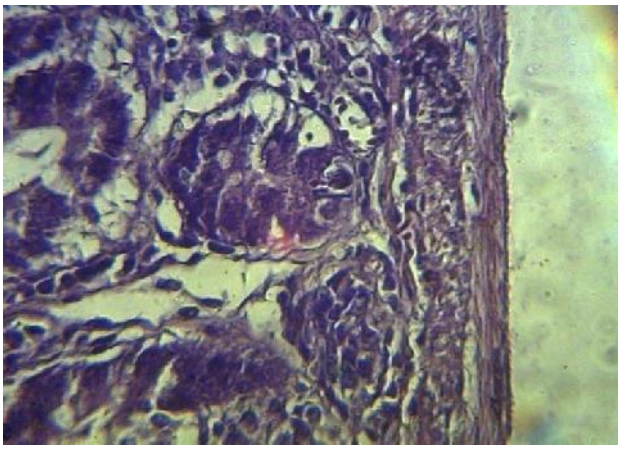


Fig 8: Histopathological section in intestine of animal 48 hours of infection with *Klebsiella pneumoniae* shows inflammatory cells infiltration in the submucosa and lamina properia of intestinal mucosa (H&EX400).

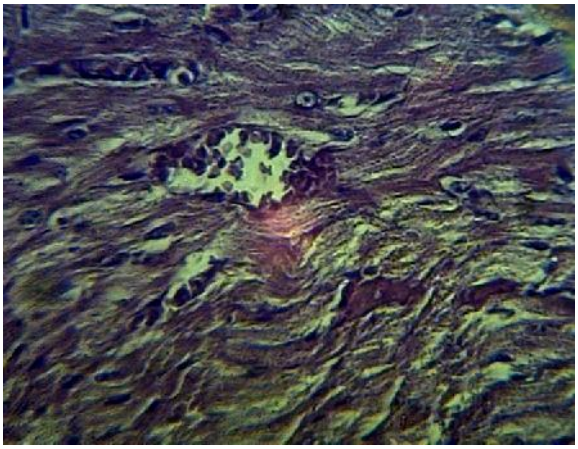


Fig 9:Histopathological section in heart of animal 48 hours infected by *Klebsiella pneumoniae* bacteria shows inflammatory cells aggregation in the lumen blood vessels and between cardiac muscle fiber (H&EX400).

Discussion

Our results showed that the rate of infection in urine samples of humane were 2%, this low rate because the duration of isolation of sample occurs in cold weather from (December / 2015 – April / 2016) this is agreement with, Khan., *et al* (2016) who recorded that the prevalence rate of *K. pneumoniae* was 1.6 times higher during the 4 warmest months of the year as compared to the rest of the year. Data suggest that rates of *K. pneumoniae* infection were associated with changes in temperature and humidity. He exact cause Citation: Seasonal Variation in *Klebsiella pneumoniae* Blood Stream Infection: for the observed higher rates of *K. pneumonia* during warm months remains elusive. Finally, *K. pneumoniae* survives better at higher humidity, as experimental models have shown that dehydration is an important factor in inactivating the organism, also Anderson,*et al* ,. (2007) that showed the rate of *K. pneumoniae* BSI and other was 1.5 times higher during the 4 warmest months of the year.

The density of *K. pneumoniae* in the environment (e.g., in freshwater ponds) is higher during warm months (Al-Harbi**,** 2003). Furthermore, the density of *K. pneumoniae* is higher in cow feces during the summer (Munoz *et al.* 2006). Thus, it is reasonable to hypothesize that humans also have higher levels of colonization with environmental *K. pneumoniae* during warm months. These environmental *K. pneumoniae* isolates are just as virulent as clinical isolates from hospitals and can produce important virulence factors (Podschun *et al*. 2001). Thus, increased colonization leading to infection may explain the observed increase in infection rate in summer.

Our result approached to that mentioned by Tu *et al*,.(2009) which referred that the infectious dose ID 50 of *Klebsiella pneumoniae* ranges between 10^2 to 10^5 CFU cells of *K. pneumonia* strain intraperitoneally or intravenously induced symptoms in mice, also Lin *et al* ,.(2009) found mice inoculated with 10^4 CFU cells of *Klebsiella pneumoniae* remained alive after 1 week, and ID is compatible with recorded the ID of *Klebsiella pneumoniae* was(2×10^4 C.F.U/ml) in mice, also Sharma *et al.*, (2011) recorded the ID of *Klebsiella pneumoniae* 10^4 CFU in mice. our results compatible with a study by Maayan *et al.*, *(*1985) showed that There was a high infection rate (85%) in mice inoculated with 10^4/ CFU of the *Klebsiella pneumoniae* that cause pathological changes in kidney of infected mice, and other internal organs.

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