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STUDY THE PATHOLOGICAL EFFECTS OF *Klebsiella pneumoniae* ISOLATED *FROM* LUNGS OF PNEUMONIA'S INFECTED SHEEP AND EXPERIMENTALLY INFECTED IN MICE.

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ABSTRACT

Currents study was designed to investigations about *Klebsiella pneumoniae* infection in sheep then evaluation its effects in mouse by experimentally infection Field study conducting on examination of one hundred100 sheep's lungs in Kerbala Province, these lungs were send to laboratory of microbes for microbial examine. Results showed 44 % of lungs samples were infected with *Klebsiella pneumoniae*; 18% of samples were negative for bacterial examination and the others 38 samples were infected with different type of bacteria but not frequently occurs as *Klebsiella e* Laboratory study, the isolated *Klebsiella pneumoniae* used to induce experimental infection in mice. Thirty 30 mixed mice at age of (4-6) weeks were used and subdivided in to three 3 group; 1st contains 10 ten males injected with 0.25ml.IP. (1.5^{*10}) suspension of bacteria; 2nd contains 10 ten females injected with 0.25ml.IP. (1.5^{*10}) suspension of bacteria; 3rd contains mixed ten mice injected with Distilled Water. Scarified 4 four animals were done at the end of one week's until last 4 four week. Samples of tissues (liver, Kidney, lung, spleen, testes, uterus) were taken from each mouse. Maine pathological lesions in lung of sheep infected with *Klebsiella pneumoniae* were suppurative broncho pneumonia, giant cell infiltration and granulomatous foci. Tissues samples of experimentally infected mice showed general abscesses at 1-2 weeks of infection, necrosis with granuloma and giant cell infiltration at 3-4 weeks of infection. Conclusion: *Klebsiella pneumoniae* infects lung sheep

and cause chronic suppurative pneumonia due to presence of giant cell as well as experimentally infects mice and causes general suppurative and granulomatous lesions.

INTRODUCTION

Klebsiella spp., particularly *Klebsiella pneumoniae*, are important causes of nosocomial infections due to the presence of capsular polysaccharide which is a major surface-located virulence properties associated with the pathogenesis of *Klebsiella pneumoniae*.(1). The capsule is an elaborate polysaccharide matrix that encases the entire cell surface and provides resistance against many host defense mechanisms. D-galacton II has an important role in synthesising the lipopolysaccharide of *Klebsiella pneumoniae* and many other gram negative bacteria.(1). *Klebsiella* species were found to be the most frequently isolated gram negative bacteria in cases of primary bacteremia (2). It is the second pathogen, next to E. coli that causes urinary tract infection. It normally affects persons with low immune system such as hospital patients, diabetes patients and people with chronic lung disease. Many a times, alcoholics also suffer from *K. pneumoniae* infections. Thus, the infections are either hospital-acquired or community-acquired (2). Characteristically, *Klebsiella* spp. produce large mucoid colonies because of the synthesis of large amounts of capsular. *Klebsiella* is one of the most important members of *Klebsiella* genus in Enterobacteriacae family, which is responsible for pneumonia (3). Besides it is found to cause infections in the urinary and lower biliary tract (4)(5).

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines(2)it can cause destructive changes to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum. In the clinical setting, it is the most significant member of the *Klebsiella* genus of the Enterobacteriaceae. *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, *Klebsiella* species have become important pathogens in nosocomial infections. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions.(3)As a free-living diazotroph, its nitrogen-fixation system has been

much-studied, and is of agricultural interest, as *K. pneumoniae* has been demonstrated to increase crop yields in agricultural conditions.(4)

Members of the genus *Klebsiella* typically express two types of antigens on their cell surfaces. The first, O antigen is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. (5)(6) Both contribute to pathogenicity and form the basis for serogrouping. It is closely related to *K. oxytoca* from which it is distinguished by being indole-negative and by its ability to grow on melezitose but not 3-hydroxybutyrate.

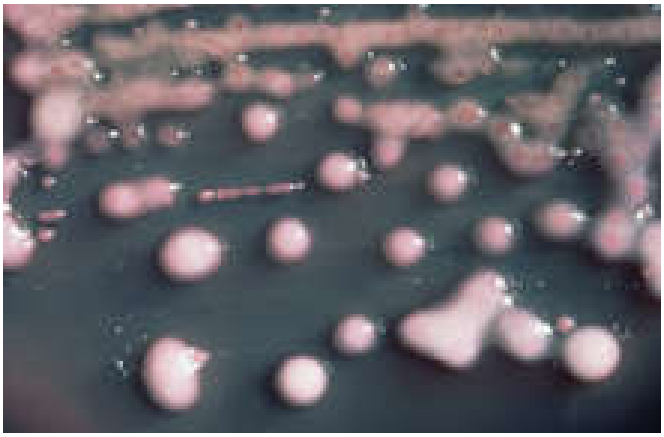


Figure (1): *K. pneumoniae* on a **MacConkey agar plate**

MATERIAL AND METHODS

Field study:

Samples: one hundred 100 lung were collected from slaughtered sheep in Kerbala Province and put in cleaned container and sent to microbial laboratory . Examination of bacteria *Klebsiella pneumonia* was doing according to authors (7).

Bacterial suspension: After examination of bacteria Bacterial Challenge Preparation: *K.peumonia* isolated from lung sample of sheep with pulmonary infection was employed in this study. Challenge dose was adjusted in mice by preparation of bacterial suspension and bacterial turbidity according to McFarland test to (1.5x10 CFU/ml).Experimental animals were infected intarperitoneally with (0.25 ml) of suitable concentration with (1.5x10 CFU)virulent viable *K.peumoniae* according to author(8)surror, 2017).

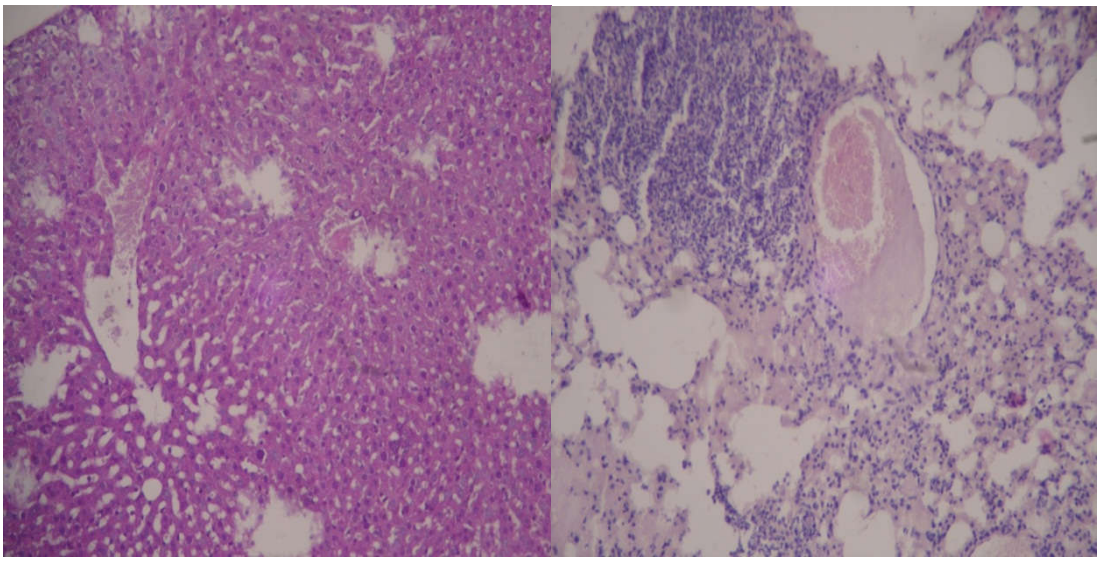
Laboratory study: White mice 30 male and female were injected with bacterial suspension at different time (72hours; 2weeks; 3weeks; 4weeks).

RESULTS AND DISCUSSION

Results of field study: microbiological examination detects that 44% of lung samples were infected with *Klebsiella pneumoniae* diagnosis performed according to authors (7)(9).

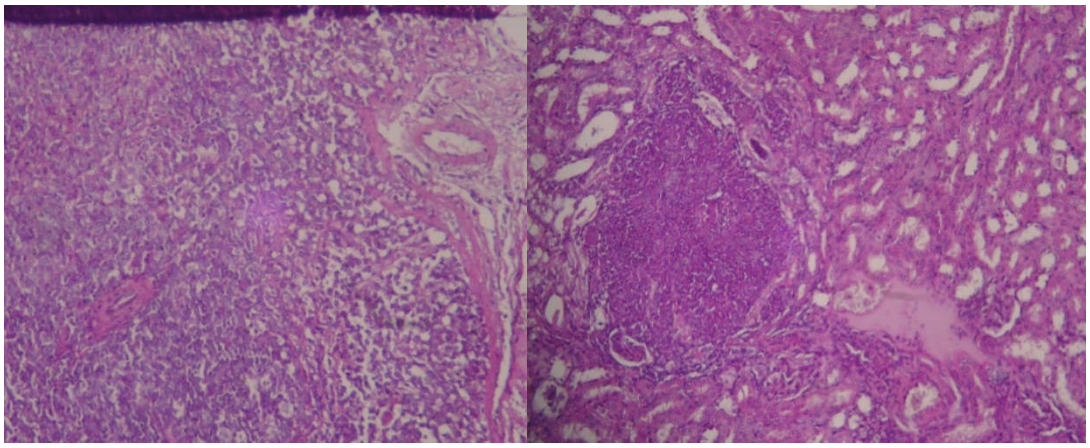
Results of Laboratory study:

A: Group 1 at 72 hours of infection: Liver showed inflammatory cells aggregation around congested central veins and in the interstitial tissue. Figure(2).Increase thickness of intra alveolar septa of lung and inflammatory cell infiltration Figure(3).



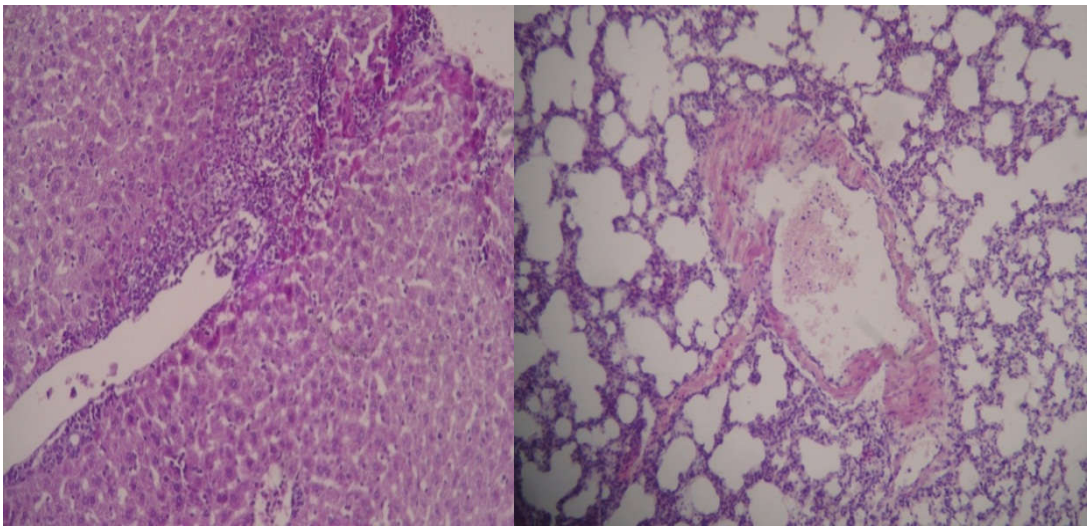
Figure(2) :Histopathological section of liver mice showing infiltration of inflammatory cell around congested blood vessels and central vein.
Figure(3) :histopathological section of lung in mice in 72 h. showing increase thickness ofInter alveolar septa and infiltration of phagocytic cell with presence of fibrinous exudation.

Spleen showed hyperplasia of endothelial cell of the central artery with hyperplasia per arterial area .Figure(4) . Kidney showed congestion of blood vessels , hemorrhage ,infiltration of inflammatory cells in the interstitial tissue, desquamation of epithelial lining cells of renal tubules ,dilatation of glomerular space .Figure(5).



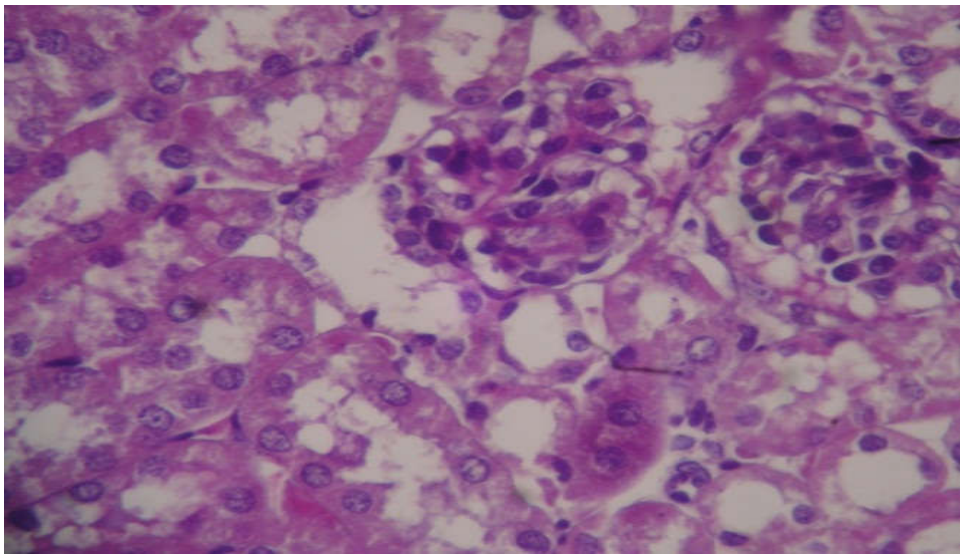
Figure(18) :Histopathological section of spleen showing inflammation around spleen
characterized by acute fibrinopurelent perisplanitis.H&E.100X.
Figure(19) :Histopathological section of kidney showing lymphocytic focal infiltration lead to atrophy of renal tubules.H&E.100X.

Group2 at 2 weeks of infection: At the period of two week of infection with *K.pneumoniae* liver showed multiple variable size micro abscess lesions around congestion of blood vessels .Figure(6). Thickening of intraalveolar septa of lung and infiltration of inflammatory cell .Figure (7).



Figure(6) :Histopathological section of liver showing micro-abscesses lesion around the central vein and scatter the liver.H&E.100X.
Figure (7) : Histopathological section of lung showing increase thickness of inter alveolar septa, congestion of blood vessels and fibroblasts proliferation.H&E.100X.

Kidney showed infiltration of inflammatory cell in the renal parenchyma, congestion of blood vessles and stenosis of renal tubules. Figure (8).



Figure(8) :Histopathological section of kidney showing infiltration of inflammatory Cell congestion of blood vessels, stenosis of renal tubules.H&E.400X.

Group 3 at 3 weeks of infection: liver showed foci granulomatous lesion. Figure (9). Lung showed increase thickness of inter alveolar septa due to excessive inflammatory reaction(10) .

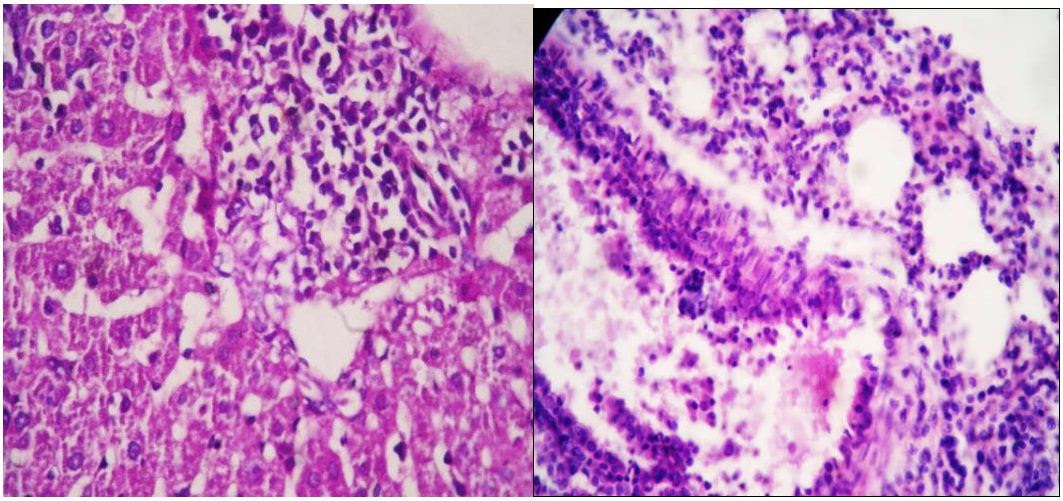
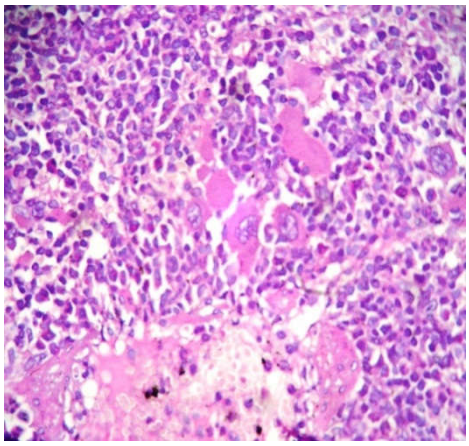


Figure (9) :Histopathological section of liver showing granulomatous lesion .H&E.400.X

Figure (10) : Histopathological section of lung showing increase thickness of inter alveolar septa from heavy inflammatory response .H&E.400.X.

Spleen showed amyloid deposition and reactive hyperplasia (11).Kidney showed necrosis, hemorrhage and atrophy of glomerular tuft .figure (12).



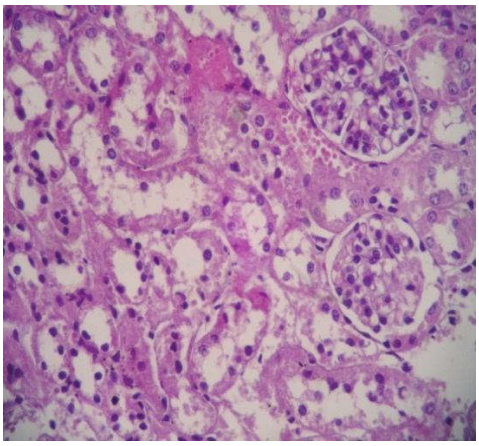


Figure (11) :Histopathological section of kidney showing necrosis, hemorrhage amyloid deposition and atrophy of glomerular tuft.H&E.400X.
Figure (12) :Histopathological section of kidney showing necrosis, hemorrhage amyloid deposition and atrophy of glomerular tuft.H&E.400X

Group4 at 4 week of infection: liver showed necrosis, hepatic cord disorganization. Figure (13). Lung showed infiltration of inflammatory cell, pervasculitis. Figure (14)

Spleen showed hypoplasia and proliferation of reticuloendothelial cell. Figure (15) .

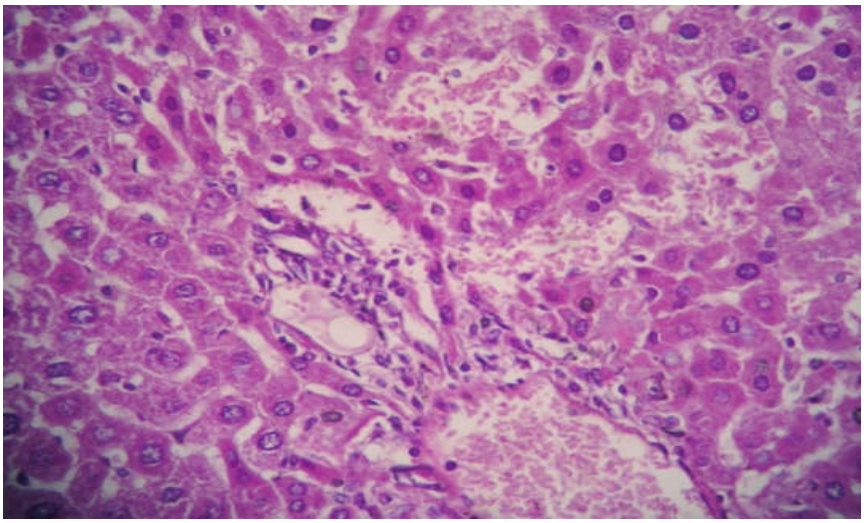


Figure (13) :Histopathological section of liver showing coagulative necrosis ; kuffer cell infiltration. H&E. 400X.

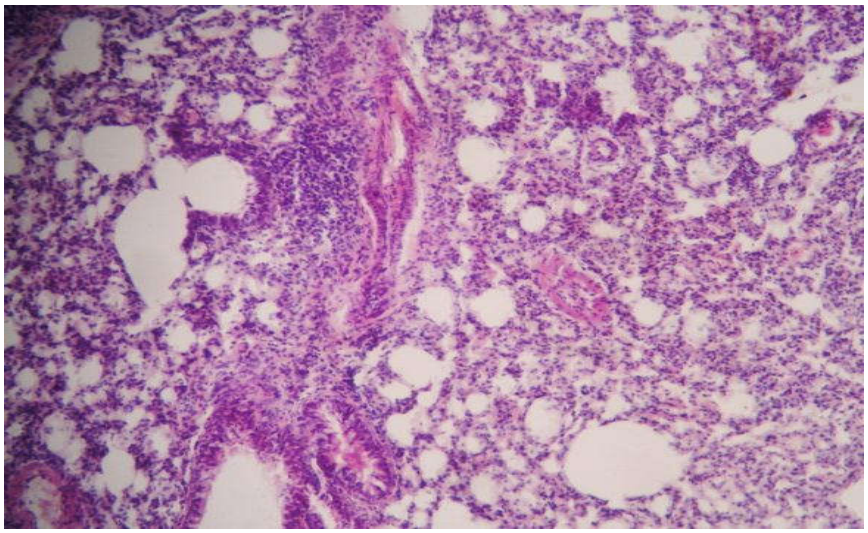


Figure (14) :Histopathological section of lung showing hyperplasia of lymphoid tissue .H&E.100 X

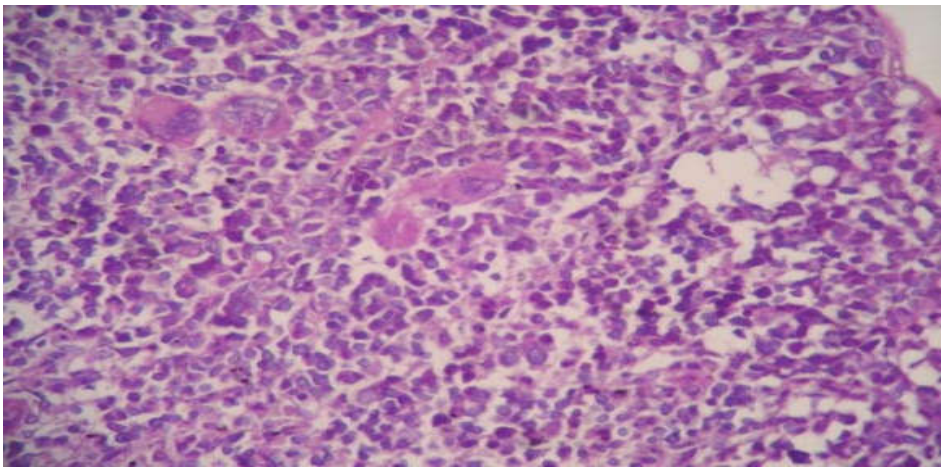


Figure (15) :Histopathological section of spleen showed amyloid deposition appear as pink area.H&E.400X.

Isolation of Klebsilla *pneumonia* from Iraqi sheep was agreed with author (10) who isolates the bacteria from ticks infects sheep living in Basrah province. But there is highly incidence of infection in lungs of sheep living in Kerbala province. Infected lungs of slaughtered sheep as well as experimentally infected mice showed interstitial pneumonia with suppurative bronchopneumonia and fibrinous pneumonia according to immune status of animals.(11). Many differences in severity of pathological finding of Pneumonia's characters in examined lungs depend on stages of infection and immunity of body against infection and virulence of microbes (12)(13)(14). Author(15) found similar finding in detection of Klebsilla *pneumonia* in Mousl city .Virulence factor of Klebsilla *pneumonia* play important role in continuously or persistence of infection(11). *K. pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during

infection(11) which acts as antimicrobial activity (16).and may cause fatal pneumonia(18).other authors revealed the same pathogen in other city in iraq(19)(20) In experimental infection in mice ,the histopathalogical change of liver after 72 hours showed inflammatory cell aggregation around the interstitial tissue and central veins, this as reported by author (21) liver parenchyma was infiltrate by large number of neutrophils associated with degeneration ,lung showed increase thickness of interalveolar septa due to infiltration of inflammatory cell this agrees with (22) who characteristics lobar pneumonia of *Klebsiella* infection had developed, also ,area of apparent cellular destruction could be visualized, as revealed by author(23) who showed histopathalogical changes like extension of poly nuclear cells through pleural membrane. At 2 weeks of infection, the histopathalogical changes of liver showed hydropic degeneration in the cytoplasm of of hepatocytes with infiltration of inflammatory cell in the parenchyma of liver and congestion of blood vessels, as said by (24) who explained occurrence mild inflammatory changes in spleen almost. The histopathalogical changes of lung showed infiltration of inflammatory cell and increase thickness of interalveolar septa, this agreed with (25) who reported sever necrosis of parenchymal tissue and infiltration of inflammatory cell. Histopathlogical changes of spleen showed hyperplasia of endothelial cells of central artery, this agreed with (26) who explained inflammatory aspects of infection in spleen.

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