Pathogenicity of *Klebsiella pneumonia* isolate from meat, meat products and Human in experimentally infected in rabbits

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Abstract

To study the pathogenicity of *Klebsiella pneumoniae* in experimentally infected rabbits, which was the second isolate after Staphylococcus sp. (26.3%), isolated from meat and meat products, collected in the period from August, 2019 to April, 2020. 18 mature local rabbits of 1-1.900 kg body weight, from both sex were used. After 2 weeks of adaptation to environment of farm of Department of Veterinary Medicine, College of Veterinary Medicine, University of Diyala, the animals divided into three groups (6 each), those of 1st group left without exposure to *Klebsiella pneumoniae*, while those of 2nd group divided into two subgroups, 1st subgroup received cortisone at therapeutic dose 0.1 mg /kg intramuscular for two times with three days interval, the 2nd subgroup received cortisone for one time at the same dose, then rabbits of this group and those of 3rd group which did not received cortisone exposed to a dose of 2x10^9 bacteria / ml., twice , three days a part, orally. The depended parameters, were the clinical, hematological and histopathological changes.

Keywords: Klebsiella, pathogenicity, rabbits

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Introduction

*K. pneumoniae* was recognized as an important food- borne pathogen in fresh products (Hamilton et al., 2006).

In recent years, Klebsiellae have become important pathogens in nosocomial infections (Alves et al., 2006). *Klebsiella pneumoniae* is one of the most important members of the genus Klebsiella in Enterobacteriaceae family, is the most medically important species, which is responsible for pneumonia (Puspanadan et al., 2012; Podschun and Ullmann, 1998). *K. pneumoniae* cause a variety of infections (Garcia et al., 1985; Ko et al., 2002). Klebsiella pneumoniae subspecies pneumonia is a common Gram- negative pathogen is a potential community- acquired pathogen causing community and nosocomial infections, including pneumonia, urinary tract, septicemia and wound infections (Podshun and Ullmann, 1998; Turton et al., 2007; Ko et al., 2002). Klebsiella pneumoniae is an opportunistic pathogen of the Enterobacteriaceae, and major hospital – acquired pathogen, and has been associated with various infections such as, wound infection, diarrheal infection, urinary tract infections and respiratory tract infections, nosocomial pneumonia, bacteremia and septicemia (Ko et al., 2002; Shubha and Ananthan, 2002; Jennifer et al., 2005). Hypervirulent *K. pneumonia* is highly invasive and can affect previously healthy persons, causing life – threatening and often community – acquired infections, such as pyogenic liver abscesses, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia (Shon and Russo, 2012; Shon et al., 2013). Bacterial pathogenicity has been shown to be due to different causes, including the structures of capsular polysaccharides (CPS; the K antigen), lipopolysaccharide (LPS; the O antigen), secreted toxins, drug resistance and genetics (Abbott, 2003; Eisenstein and Zaleznik, 2000; Podschun and Ullman, 1998; Cheng et al., 2000; Cheng et al., 1991). Capsule plays a very important role in virulence (Sikarwar and Batra, 2011). The aims of current study was study the pathogenicity of Klebsiella pneumoniae in experimentally infected rabbits

Material and methods

Eighteen mature local rabbits of 1-1.900 kg body weight, from both sexes were used. After 2 weeks of adaptation to environment of farm of Department of Veterinary Medicine, College of Veterinary Medicine, University of Diyala, the animals divided into three groups (6 each), those of 1st group left without exposure to Klebsiella pneumoniae, while those of 2nd group divided into two subgroups, 1st subgroup received cortisone at therapeutic dose 0.1 mg /kg intramuscular for two times with three days interval, the 2nd subgroup received cortisone...
for one time at the same dose, then rabbits of this group and those of 3rd group which did not received cortisone exposed to a dose of 2x10⁹ bacteria / ml., twice , three days a part, orally.

The depended parameters, monitoring the animals to record any changes in behavior, appetite, feces or any signs, together with clinical parameters (Body weight, Body temperature, Heart rates and Respiratory rates), were done according to (Radostits, et al., 2007); Hematological, included (Hgb concentration, and HCT% ; Total and Differential leucocytes counts) according to (Coles, 1998) were done at starting of experiment, 6th day of experiment, and at a time of sacrificed of animals, with the help of ether as anaesthetic agent. Each animal were examined grossly to record the gross changes in animals viscera. A samples from gastro-intestinal, heart, lungs, kidney, liver were collected for histopathological examination, (hematoxylin - Eosin stain) according to ( Luna and Lee,(1968).

**Statistical analysis**

Statistical analysis of data was performed using ANOVA, unpaired t test and lowest significant differences (LSD). All experimental data are presented as Mean ±S.E. The results were considered significant if P < 0.05. (Steel et al., 2007).

**Results:**

*Klebsiella spp.,* was the highest isolates from workers, and their equipment (44/175 (25.1%), then from sheep and cow 13/35 (37.1%), and poultry 9/41 (22.0%). (Table-1-).

<table>
<thead>
<tr>
<th>Origin</th>
<th>No.</th>
<th>Kl.</th>
<th>Ps.</th>
<th>Sta.</th>
<th>E</th>
<th>Sal.</th>
<th>List</th>
<th>Pro</th>
<th>Citr</th>
<th>Str</th>
<th>Yer</th>
<th>Sh</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workers</td>
<td>175</td>
<td>44</td>
<td>26</td>
<td>42</td>
<td>34</td>
<td>4</td>
<td>3</td>
<td>14</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>197</td>
</tr>
<tr>
<td>Sheep, beef</td>
<td>35</td>
<td>13</td>
<td>9</td>
<td>19</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Poultry</td>
<td>41</td>
<td>9</td>
<td>9</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
<td>66</td>
<td>44</td>
<td>77</td>
<td>50</td>
<td>19</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

From 251 samples, (328) isolates were isolated. The highest number of isolates was *Staphylococcus* sp. (30.7%), followed by *Klebsiella* sp. (26.3%), *E. coli* (19.9%), *Pseudomonas* sp. (17.5%), *Salmonella* sp. (7.6%), *Listeria* sp. (6.4%), *Proteus* and *Citrobacter* each (5.6%), *Enterobacter* (5.2%), *Yersinia* sp. (3.2%), *Streptococcus* (2.4%) and *Shigella* sp. (0.4%)

**Clinical signs**

The animals exposed to *Klebsiella pneumoniae* exhibits signs of anorexia, depression, dyspnea, and engorged blood vessels, three of them died. Heart rates increased in GII and GIII. Respiratory rates increased in GIII, body temperature non significantly increased in GIII, body weight non significantly decreased in GIII.(Table -2-)

<table>
<thead>
<tr>
<th>parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>H.R./min</td>
<td>142.5±3.23a</td>
<td>143.75±3.84a</td>
<td>140.5±1.10a</td>
</tr>
<tr>
<td>R.R./min.</td>
<td>131.25±10.08a</td>
<td>136.25±5.54a</td>
<td>130.5±6.50a</td>
</tr>
</tbody>
</table>

**Table -1- Total isolates which were isolated in current study**

**Table -2- Heart rates/min, Respiratory rates/min., Body temperature °C, body weight kg. of rabbits used in study**

Values are M S.E., a, b significantly difference at P<0.05

Hematological changes

Total leucocytes counts significantly increased in GII and GIII. Basophiles % no significant changes. Monocytes significantly increased in 3rd reading in GII. Eosinophiles % no significant changes. Lymphocytes % increased significantly in GII. Neutrophils increased in GII. PCV increased in GIII, no significant changes in hemoglobin. (Table -3-)

Table -3- hematocrit, hemoglobin concentration, total leucocytes count, and differential leucocytes of rabbits used in study

<table>
<thead>
<tr>
<th>parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Hgb g/dl</td>
<td>12.65 ± 0.31a</td>
<td>12.53 ± 0.38a</td>
<td>12.95 ± 0.69a</td>
</tr>
<tr>
<td>PCV%</td>
<td>34.5 ± 2.33a</td>
<td>36.0 ± 1.08a</td>
<td>37.25 ± 2.02a</td>
</tr>
<tr>
<td>T.L.C.x10³/ cmm</td>
<td>9.175 ± 4.68a</td>
<td>8.375 ± 0.90a</td>
<td>9.500 ± 2.0a</td>
</tr>
<tr>
<td>Lymph.%</td>
<td>47.0 ± 2.50a</td>
<td>44.25 ± 3.0a</td>
<td>48.0 ± 5.0a</td>
</tr>
<tr>
<td>Neutroph. %nd</td>
<td>45.6 ± 1.15a</td>
<td>46.75 ± 2.0a</td>
<td>44.5 ± 3.0a</td>
</tr>
<tr>
<td>Eosinoph.%</td>
<td>3.2 ± 0.75a</td>
<td>3.5 ± 0.25a</td>
<td>2.75 ± 0.75a</td>
</tr>
<tr>
<td>Basoph.%</td>
<td>1.2 ± 0.1a</td>
<td>0.75 ± 0.25a</td>
<td>1.25 ± 0.5a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.2 ± 1.30a</td>
<td>3.75 ± 1.20a</td>
<td>3.5 ± 0.80a</td>
</tr>
</tbody>
</table>

Values are M S.E., a, b significantly difference at P<0.05

Postmortem findings

Three of rabbits were died during the experiment. The other were sacrificed at the end of experiment. One animal that received two doses of hydrocortisone at a dose of 0.1 mg intramuscular twice with three days apart before exposure to K. pneumoniae at a dose of 2x10⁹/ml two milliters orally, died in the 2nd day post exposure to bacteria. The main gross lesion were sever congestion with presence of fluid and un clotted blood in lungs, congestion and enlargement of heart, enlargement and congestion with foci of abscess in liver, patchy hemorrhages on gastric mucosa, sever congestion of gastrointestinal mucous membranes, enlarged and congestion of kidneys with retention of urine.
The second animals from the same group and exposed as the first above animal died three days post exposure to bacteria. The gross lesion were, flabby, enlarged severely congested heart, congestion of lung, liver enlarged. Congested and liver abscess, patchy hemorrhages on gastric mucosa, enlarged and congested kidneys.

The third animals died 7 days post exposure to bacteria, but not received cortisone. the main gross lesions were congestion of heart, lungs, enlarged and congested liver, patchy hemorrhages of gastric mucosa, enlarged and congested.
Pictures A: clear postmortem findings: congestion, engorged blood vessels, in heart, lungs, liver, gastrointestinal tract, retention of urine in urinary bladder, pneumonia, enlarged heart, kidneys. Liver abscess.

Pictures B: clear postmortem findings: congestion, engorged blood vessels and hemorrhage, in heart, lungs, liver, gastrointestinal tract, retention of urine in urinary bladder, pneumonia, enlarged heart, kidneys, liver.

Histopathological changes

Third animal which died 7 days post exposure to $2 \times 10^9$ /ml, 2 mls, twice times, three days apart, not received cortisone: exhibit; necrosis in lungs, destruction of alveoli, hemorrhages, kidneys showed proliferation of inflammatory cells (neutrophils), with a few of mononuclear cell (lymphocytes and mesangial cells. Figures (1-2).
Figure (1): Lung showed necrosis (←), distraction of the alveoli (→) and hemorrhage (↓), this refer to the necrotic hemorrhagic pneumonia. (H&E stain 40X).

Figure (2): Kidney showed proliferation of inflammatory cells (neutrophils) with a few of mononuclear cells (lymphocyte and mesangial cells) (←), also there is area of coagulation (↓), this case refer to the coagulative nephritis. (H&E stain 40X).

Animal that died, received a dose of hydrocortisone, 0.1 mg I.M. twice times three days apart, exposed to $1 \times 10^6$ *K. pneumoniae* /ml, 2 ml. orally. The main gross lesions were found in the liver which showed dead necrotic area with the fatty vacuoles stomach showed amyloid deposition in the lamina propria with hyperplasia in mucosal gland. Heart showed proliferation of fibroblastic cells in the myocardium result in fibrosis. Figure (3-5).
Figure (3): Liver showed dead necrotic area (←→) with the fatty vacuoles (←→). (H&E stain 40X).

Figure (4): Stomach showed amyloid deposition in the lamina propria (←→) with hyperplasia in mucosal gland (←→). (H&E stain 40X).

Figure (5): Heart showed proliferation of fibroblastic cells in the myocardium result in fibrosis. (H&E stain 40X).
Animal sacrificed, received one dose of hydrocortisone, 0.1 mg I.M., and exposed to bacteria at a dose of $2 \times 10^9$ /ml, 2 ml, twice, three days apart. The main gross lesions were, lung showed proliferation of the inflammatory cells neutrophils with a few mononuclear cells, there is expansion of alveoli and destruction of the alveolar walls, this refer to pulmonary emphysema. Amyloid present in some area of the lung, small intestine showed hemorrhage nearly the microvilli of intestine, and presence of vacuolation in submucosal layer, this result in hemorrhagic enteritis. Liver showed hepatocyte hyperplasia, and presence of fatty changes Figures (6-8).

Figure (6): Lung showed proliferation of the inflammatory cells neutrophils with a few mononuclear cells, there is expansion of alveoli and destruction of the alveolar walls, this refer to pulmonary emphysema. Amyloid present in some area of the lung. (H&E stain 40X).

Figure (7): Small intestine showed hemorrhage nearly the microvilli of intestine and presence of vacuolation in submucosal layer, this result in hemorrhagic enteritis. (H&E stain 40X).

Figure (8): Liver showed hepatocyte hyperplasia and presence of fatty changes. (H&E stain 40X).

Animals died, received one dose of cortisone 0.1 mg IM and exposed to Kl. pneumoniae at a dose of $1 \times 10^6$ /ml, 2 ml, twice three days a part. The main histopathological lesions were, lung showed expansion and severe distraction of alveoli, hemorrhage in alveolar wall and thickness of alveolar wall in some area, kidney showed kupffer cells a rounded glomeruli with the fatty vacuoles Figure (9-10):
Figure (9): Lung showed expansion and sever distraction of alveoli (→), hemorrhage in alveolar wall (←) and thickness of alveolar wall in some area (↔). (H&E stain 40X).

Figure (10): Kidney showed kupffer cells a rounded glomeruli (→) with the fatty vacuoles (←). (H&E stain 40X).

Discussion

The results of current study, the animals were isolated, anorexic, depressed, signs of pneumonia, three of animals exposed to bacteria were died, the heart rate and respiratory rates increased as a result of infection, total leucocytes increased also. Others parameters either no changes or non-significantly changed. This can attributes to duration and dose of exposure.

The symptoms of the disease characterized by fatigue, anorexia, nausea, diffuse abdominal discomfort, pleuritic chest pain, jaundice and fever (lederman and Grum, 2005).

*K. pneumonia* bacteremia causes significant morbidity and mortality if incorrectly treated in general populations (Tsai *et al*., 2010).

The result of pathogenicity of Klebsiella spp. revealed that the clinical signs, showed body weight was decreased, prolonged bleeding time, prolonged clotting time. Hemoglobin concentration, PCV percentage and erythrocytes count were decreased. ALT level increased AST less changes total serum bilirubin level were increased. Total leucocytes counts were increased (Abeer 2017).
Grossly, the changes in those died or sacrificed at end of experiment, showed congestion in lung, heart, liver and kidneys enlarged and congested, hemorrhages on gastric mucosa, with severe congestion, with abscess formation in liver. Histologically lungs showed alveolar destruction with hemorrhages, kidney showed infiltration of inflammatory cells mainly neutrophils, with few mononuclear cells (Lymphocytes). in addition to areas of coagulation (coagulative nephritis).

The K1 capsular serotypes are the predominant serotype of *K. pneumoniae* strains causing liver abscess (Struve et al., 2005).

Histopathologically lesions included infiltration of inflammatory cells within heart, liver, stomach, lung and kidney, congestion of blood vessels of liver and lung, kidney degeneration and liver fatty degeneration, damage of alveolar wall of lung. (Abeer 2017).

*K. pneumonia* is an opportunistic and major hospital – acquired pathogen, causing urinary tract infections, nosocomial pneumonia, bacteremia and septicemia (Ko et al., 2002). *K. pneumoniae* is a potential community-acquired pathogen (Ko et al., 2002). Community – acquired pneumonia is a very severe fatal illness with a rapid onset, high fever, and haemoptysis (current jelly sputum), bulging interlobar fissure and cavity abscesses observed by chest radiographic.

*Klebsiella pneumoniae* subspecies pneumonia is a common Gram- negative pathogen causing community and nosocomial infections, including pneumonia, urinary tract, septicemia and wound infections (Podshun and Ulmann, 1998; Turton et al., 2007).

*K. pneumoniae* is also an opportunistic pathogen, capable of causing a wide range of diseases in humans and other animal species. *K. pneumoniae* is most notorious for causing extraintestinal human infections such as pneumonia, cystitis, pyelonephritis, septicemia, and pyogenic liver abscess (Podshun and Ulmann, 1998; Laupland et al., 2007; Shon et al., 2013). Similarly, *K. pneumoniae* causes a wide array of extraintestinal infections in other animal species (Du et al., 2014 and Brisse and Duijkeren, 2005).

*Klebsiella pneumoniae* is an opportunistic pathogen of the Enterobacteriaceae, and major hospital – acquired pathogen, and has been associated with various infections such as, wound infection, diarrheal infection, urinary tract infections and respiratory tract infections, nosocomial pneumonia, bacteremia and septicemia (Ko et al., 2002; Shubha and Ananthan, 2002; Jennifer et al., 2005).

Klebsiella can cause infections of respiratory tract, nasal mucosa, pharynx and generally results in primary pneumonia. Pneumonia is the most frequent nosocomial infection (30 to 33%) of cases among combined medical surgical intensive care units participating in the National Nosocomial Infections Surveillance System (Richards et al., 2000).

*K. pneumoniae*, a member of the human intestine flora, is frequently associated with hospital-acquired pathogen causing severe respiratory tract infections such as pneumonia. Other infections caused by this organism include urinary tract infections, wound infections, abscesses, sepsis, infections of the blood stream, inflammation and diarrhea (Hackstein et al., 2013).

*K. pneumonia* strains exhibit different virulence factors which give the bacteria the ability to invade the host, including the structures of capsular polysaccharides (CPS; the K antigen), lipopolysaccharide (LPS; the O antigen), secreted toxins, drug resistance and genetics, serum resistance, production of urea and enterotoxin, type 1 and type 3 adhesions, factors involved in aggregative adhesions and siderophores (Damian et al., 2009; Abbott, 2003; Eisenstein and Zaleznik, 2000; Chang et al., 2000; Podshun and Ulman, 1998; Cheng et al., 1991). Capsule plays a very important role in virulence (Sikarwar and Batra, 2011).

In particular, serotype K1/ K2 – caused pyogenic liver abscess, often complicated by metastatic infections, has emerged worldwide in the past two decades (Siu et al., 2012). The ability to metastatically spread from one organ to other organs is characteristics of hypermucoviscous *K. pneumoniae*.

Hypervirulent *K. pneumoniae* is highly invasive and can affect previously healthy persons, causing life – threatening and often community – acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia (Shon and Russo, 2012; Shon et al., 2013).
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