Expression of Caspase-3 in the Liver and Spleen *Rattus norvegicus* Which Infects Bacteria *Klebsiella pneumonia* and *Klebsiella pneumoniae* Extended Spectrum Beta Lactamase

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Keywords: Apoptosis, Caspase-3, *Klebsiella pneumoniae, Klebsiella pneumoniae* ESBL.

Abstract: *Klebsiella pneumoniae* (*K. pneumoniae*) is found in nosocomial infections and Gram-negative number two is the most dangerous. The bacteria *K. pneumoniae* causes unusual nosocomial infections in hospital settings. Drug resistance in bacteria *K. pneumoniae* ESBL production may increase the risk of death at the time of treatment. Caspase-3 is the hallmark of apoptosis. It can be used as an indicator of virulence of the infection of *K. pneumoniae*. This research aims to look at the effects of infection of *K. pneumoniae* and *K. pneumoniae* ESBL against eksprsi caspase-3 in the liver and spleen of rats *Rattus novergicus*. The study uses 12 healthy rats that were divided into three groups, namely the control group, infection *K. pneumoniae,* and infection *K. pneumoniae* ESBL. Each group of samples taken from the liver and spleen organs after 24 hours of the infection process and immunohistochemical expression to see caspase-3. Of the research results obtained, the percentage of caspase-3 expression in the liver and spleen organs in rats that infect bacteria *K. pneumoniae,* are higher than *K. penumoniae* ESBL. The expression of caspase-3 in the liver and spleen organs is steeper than the infection of *K. pneumoniae*. We conclude that the bacteria *K. pneumoniae* is more virulent than *K. pneumoniae* ESBL.

1 INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is found in nosocomial infections and Gram-negative number two is the most dangerous (Tsai et al., 2009). The bacteria *K. pneumoniae* causes unusual nosocomial infections in hospitals (Woldu, 2016). The bacteria *K. pneumon*iae is very dangerous for patients with immunocompromised and immunodeficiency, causing damage to organs (Wu, 2015). The transmission of *K. pneumoniae* in hospitals has become a particular concern in Europe, the United States, Argentina, and Australia. Infections caused by *K. pneumoniae* result in longer treatment times in hospital and resistance to antibiotics (Brisse et al., 2006).

Extended Spectrum Beta Lactamase (ESBL) *K. pneumoniae* produces an aggravating risk factor for infection. The bacteria *K. pneumoniae* ESBL production is resistant to beta-lactam antibiotics and are at risk of adding to long treatment times in ICUs (Toner et al., 2016). The virulence of *K. pneumoniae* this decade can infect normal or healthy individuals, due to resistance to drugs and hipervirulen (Paczosa, 2016)

Apoptosis plays a role in bacterial infection. Apoptosis affects the immune cells that are very important in the course of the infection. Regulation of apoptosis is an important aspect of the host cell response to stress and infection and should be continual. Caspase in the apoptosis process plays a role in cellular processes, which occur before apoptosis happens (Silva, 2009). Apoptosis triggered by the TNFα is produced by inflammation from the bacterial infection Klebsiella pneumuniae good through the intrinsic or extrinsic pathways triggered by various cellular responses through the endotoxin lipopolysaccharide (LPS). The Endotoxin bacteria *K. pneumuniae* often triggers endothel cell damage so multiple organ failure can occurs. Endotoxin triggers the inflammatory process, which is sustainable and increases the incidence of cell death in the follicle cell dendritic, dendrite cells, neutrophils, $CD4^+$,

Andika Sukarya, I., Sandhika, W. and Wahyu Widodo, A.
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DOI: 10.5220/0007540702440248
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 244-248
ISBN: 978-989-758-348-3

$CD8^+$, and B cells (Paczosa, 2016). Caspase-3 is a death protease activation of mediators that frequently programs cell death (apoptosis), and catalyzes the cleavage of cell specific proteins of some. Caspase-3 is the hallmark of apoptosis, and indispensable for the condensation of the cell apoptosis and fragmentation of DNA. Caspase-3 is very important in the process of apoptosis (Porter, 1999).

The spleen immune system is responsible for protecting the body from the invasion of pathogens and detecting old cells, damaged mechanically, and distorted, which can lead to the formation of tumors. The spleen plays out a double simultaneous reaction against bacterial antigens and allogenes. As a secondary lymphatic system, due to the number of lymphocytes in the spleen area, more and bacteria will be taken towards the organ spleen. Lymphocytes in the spleen undergo apoptosis. The liver is the largest location of kupffer cells, NK cells, and NKT cells. Cells, NK cells and kupffer NKT cells are activated by the stimulation of APC, both directly and indirectly, to respond to bacterial infections. Activation of the cells that are in the liver apoptosis are triggered to respond to infections (Wang, 2014). The expression of caspase-3 denotes prosen apoptosis, which occurs during the ongoing infection process (Porter, 1999).

In this study, we will present the expression of caspase-3 in infection *K. pneumuniae* and *K. pneumuniae* production of ESBL in Rattus norvegicus. Expression of caspase-3 in the liver and spleen of a rat show virulence infection *K. pneumuniae* and *K. pneumuniae* ESBL. This can be seen with the virulence of the infection *K. pneumuniae* and *K. pneumuniae* in the liver and spleen organs.

2 MATERIALS AND METHODS

2.1 Animals

The rat *Rattus novergicus*; male rats that are healthy and not exposed to infection. They are characterized by the movement of agile rats, aged three months, with a weight of 200–250 grams.

2.2 Bacteria

The bacteria *K. pneumoniae* ESBL and *K. pneumoniae* derived from Installations Clinical Microbiology of Dr. Sutomo Surabaya. Made with 10^5 CFU of bacteria concentrations of Phosphate-buffered saline (PBS).

2.3 Method

The healthy rat is selected by raffled and is given a mark or code of any group consisting of four rats. Injection materials on the peritoneum in Quadrant 3 included as much as 1ml (Group control in injection PZ (Phisiological zouth), a group of *K. penumoniae* injection on concentrations with 10^5 CFU *K. pneumoniae*, and groups of *K. pneumonie* ESBL injection on concentrations with 10^5 CFU *K. penumonie* ESBL) in each group of animals. The rats were observed for 24 hours. The livers and spleen organs were retrieved 24 hours after surgery. The rat's liver and spleen fixation buffer into the tissue preparation and formaldehyde (formalin fixed and paraffin embedded section). The sample was made of paraffin blocks and cutting samples with a thickness of 4µm, using either a microtome or tool placed on the microscope slide. An immunostaining process was performed using the immunohistochemistry reagents Caspase-3 (Bioss Antibodies, Bioss, USA).

2.4 Immunohistochemistry

Deparaffinization and rehydration**;** Wash slides twice in Xylene for three minutes each time in real time; Wash slides in Xylene 1:1 with 100% ethanol for three minutes in real time; Wash slides twice in 100% ethanol for three minutes each in real time; Wash slides twice in 95% ethanol for three minutes each in real time; Wash slides in 70% ethanol for three minutes in real time; Wash slides in 50% ethanol for three minutes in real time; Rinse slides gently with running distilled water for five minutes in real time. Antigen retrieval**;** Boil slides in 0.01M sodium citrate buffer (pH6) at 100°C for 15–20 minutes; Remove the slides from the heat and allow them to stand at real time in the buffer for 20 minutes; Rinse twice with TBST for five minutes in real time. Immunostaining**;** block with endogenous peroxidase with 3% hydrogen peroxide for 30 minutes; block with 5% serum or BSA for two hours in real time; drain blocking buffer from slide; incubate slides with the diluted primary antibody overnight at 4°C with gentle agitation; Wash slides twice with TBST for five minutes in real time; incubate slides with diluted conjugated secondary antibody for two hours in real time with gentle agitation; wash slides twice with TBST for five minutes in real time; develop with chromogen for 10

minutes in real time; wash slides in distilled water for one minute in real time; counterstain (if required); Dehydrate when using a chromogen substrate that is alcohol insoluble by washing slides in 80%, 95%, 100%, and Xylene each for one minute in real time; Mount coverslips; check out the slides and calculate the percentage of positive cells caspase-3 there are liver and lymphatic organs.

3 RESULT

The research results were obtained as a percentage of the number of cells that undergo caspase-3 on the organ spleen and liver in the treatment group infection *K. pneumoniae*, *K. pneumoniae* ESBL and the control group (Figures 1 and 2).

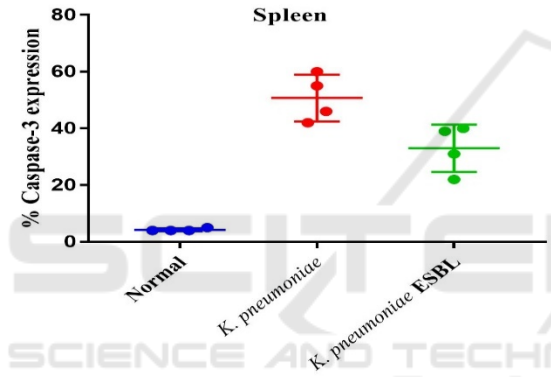

Figure 1: Box-plots the percentage of cells that undergo caspase-3 in the spleen.

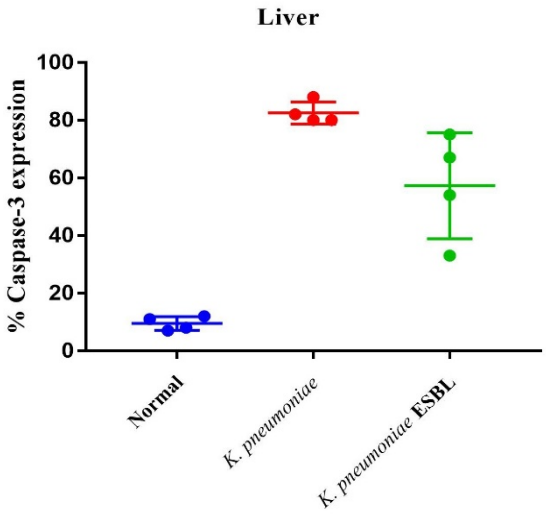

Figure 2: Box-plots the percentage of cells that undergo caspase-3 in the liver.

Figure 1 shows the percentage of caspase-3 in the lymphatic organs. The group treatment in infection *K. pneumoniae* are higher than group treatment on infection of *K. pneumoniae* ESBL and those in the control group. The value of the median is the highest to the lowest in succession, i.e. the Group of *K. pneumoniae* (45.5), *K. pneumonia* ESBL (35), and the control group (4).

Figure 2 shows the percentage of caspase-3 in the liver organ on group infection *K. pneumoniae* in treatment was higher in the treatment group of infection *K. pneumoniae* ESBL and those in the control group. The value of the median is the highest to lowest in succession i.e., the Group of *K. pneumoniae* (81), *K. pneumonia* ESBL (60.5) and the control group (9).

Figure 3 shows *R. norvegicus* spleen cells demonstrating the immunostaining Caspase-3; The control group is shown in picture A with a magnification microscope 1000x; *K. pneumoniae* group is shown in picture B with a magnification microscope 1000x. There are many cells that express caspase-3, such as arrows. Labelled cells in the spleen are experiencing the process of apoptosis, and groups of *K. pneumonia* ESBL in picture C shows the cells at 1000x magnification. On the organ in a bacterial infection *K. pneumoniae* and *K. pneumoniae* ESBL shows the expression of caspase-3 on the cell. This marks the process of apoptosis occurring on the spleen, caused by a bacterial infection *K. pneumoniae*, causing almost 60% of damage to the spleen organ.

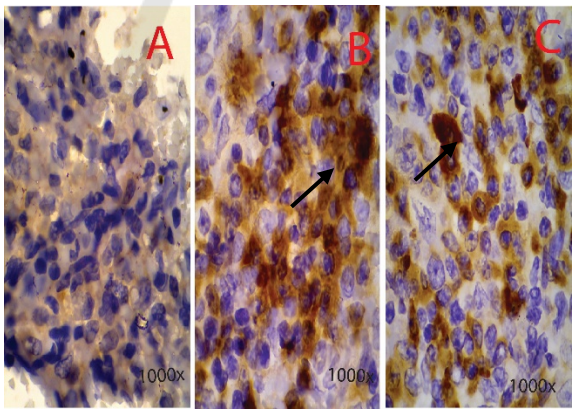

Figure 3: Cells in the spleen tissues of *R. norvegicus* in immunostaining caspase-3 in the control group Figure A, *K. pneumoniae* Group Figure B and *K. pneumonia* ESBL Figure C.

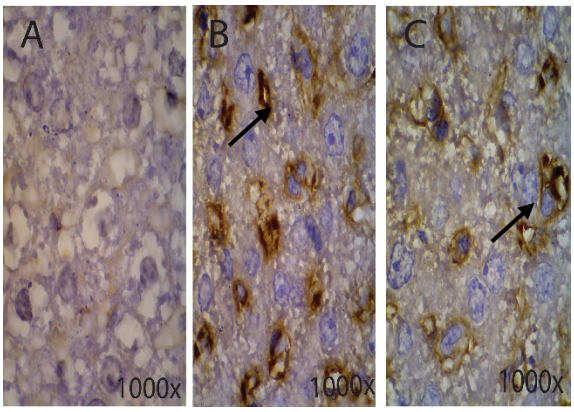

Figure 4: Cells on *R. norvegicus* in liver tissue immunostaining caspase-3 in the control group Figure A, , *K. pneumoniae* group Figure B and *K. pneumoniae* ESBL group Figure C.

Figure 4 shows *R. norvegicus* demonstrating the liver tissue in immunostaining caspase-3; the control group is shown in picture A at 1000x magnification using a microscope; the *K. pneumoniae* group is shown in picture B at 1000x magnification using a microscope. There are many cells that express caspase-3, such as arrows and labelled cells in the liver that are experiencing the process of apoptosis. For the *K. pneumonia* ESBL group, picture C shows cells at 1000x magnification using a microscope. On the organ in a bacterial infection, *K. pneumoniae* and *K. pneumoniae* ESBL shows the expression of caspase-3 on the cell. This marks the process of apoptosis occurring in liver organs caused by the bacterial infection *K. pneumoniae*, causing 80% liver organ damage.

4 DISCUSSION

There was an increase in the percentage of caspase-3 in liver and spleen organs in a group of animal models with the infections *K. pneumoniae* and *K. pneumoniae* ESBL. Injection of germs in an animal model is done through the peritoneal line. The test compound injected into the peritoneal cavity will be absorbed into the portal circulation and transported to the liver (Shayne et al., 2013). The liver receives the blood vein of the portal and the arterial blood, liver, and spleen are important components in the defense against infection entering the blood stream. To achieve this role, the liver and the spleen contain many innate and adaptive immune cells specifically to detect and capture pathogens from the blood stream. Furthermore, the immune cells participate in the immune response that leads to the purge of pathogens, the recruitment of leukocytes and antigen presentation to lymphocytes in the blood stream. An increasing number of caspase-3 in liver organs can be caused due to a high inflammatory process, which triggers the cells to hepatocyte or innate underwent apoptosis. There is a balance between activation and tolerance that characterizes the liver and spleen as immunological organ front line (Jenne and Kubes, 2013).

The spleen is an organ of the lymphatic system and inserted into the bloodstream is a collection of lymphoid tissues. The spleen immune system is responsible for protecting the body from the invasion of pathogens and detecting old cells, damaged mechanically and distorted that can lead to the formation of tumors. Recent studies prove the dominant role in the simultaneous double reaction against bacterial antigens and allergens. The spleen is the seat of an innate and adaptive immune system. Microbial network penetration evokes an innate system direct reaction, while the adaptive immune response involves the interaction of cells that recognize specific antigens in the context with the MHC presented by a cell that gives rise to antigens (Wluka et al., 2006) A secondary lymphatic system allows the number of lymphocytes in the spleen area to increase, and infected bacteria is carried towards the spleen organ. Lymphocytes in a lien allows will undergo apoptosis. Of the function that has been described above, an increasing number of caspase-3 in liver and spleen organ can be caused due to the high inflammatory process to trigger the cells to hepatocyte or undergoing innate apoptosis.

Caspase-3 positive cells in the spleen and liver organs in animal models with the infection of *K. pneumoniae* are higher than animal models in infection of *K. pneumoniae* ESBL. The bacterium *K. pneumoniae* and *K. pneumonie* ESBL triggered a wide range of cellular response through the endotoxin lipopolysaccharide (LPS). The ongoing process of inflammation triggers endotoxins and increases the incidence of apoptosis on dendrite cells, follicular dendritic cells, and neutrophils nanotechnologies, the number of $CD4^+$, $CD8^+$ and B cells (Paczosa, 2016). *K. pneumoniae* protects a variety of humoral defense mechanisms, such as the mechanism of bacterial resistance to complement the damage. In phagocytosis, this did not happen on *K. pneumoniae* ESBL. Human beta-defensins 1 (HBD-1) and HBD-2 inefficient kill *K. pneumoniae* as HBD-3. *K. pneumoniae* ESBL production is more susceptible to HBD (Moranta et al., 2010). Caspase-3 indicates that high apoptosis occurrence is very high and is prominent regarding pathological liver

disease. The end result of caspase-3 is the high cause of dysfunction of the liver, cirrhosis, and tumorigenesis (Wang and Lien, 2013).

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