

# Differences of Caspase-3 Expression in the Spleen and Liver of Sepsis Models in Rats Infected with *Escherichia coli* ESBL and *Klebsiella pneumoniae* Carbapenemase

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**Abstract :** Sepsis is the leading cause of death in the world. Sepsis patients with Extended Spectrum  $\beta$ -lactamase (ESBL)-producing bacterial infections were 57.4% *Escherichia coli*, 21.35% *Enterobacter* sp, and 21.3% *Klebsiella* sp. Caspase-3 is the most important caspase effector responsible for morphological and biological changes in apoptotic cells. This type of research is true experimental with a post-test only control group design, using one control rat group and two groups of rats infected with *E. coli* Extended Spectrum  $\beta$ -lactamase (ESBL) and *Klebsiella pneumoniae* carbapenemase (KPC) for 24 hours to find out the different expression of caspase-3 in the spleen and liver of those infected rats. Expression of caspase-3 was observed by staining the spleen and liver with caspase-3 p12 subunit antibody. Cells expressing caspase-3 were counted under the light microscope. The results showed that caspase-3 expression in the KPC infected spleen group was 65.25 $\pm$ 12.69%, whereas *E. coli* ESBL was 33.75 $\pm$ 3.862%. This is thought to be influenced by the presence of antigen differences between the two bacteria, thus the possibility of apoptosis in lymphocyte cells caused by KPC would be higher when compared with those infected with *E. coli* ESBL. Caspase-3 liver expression in the KPC group had a value of 58.75 $\pm$ 4.031%, while the *E. coli* ESBL infected was 48.75 $\pm$ 6.292%. It may be affected by differences in soluble factors of both bacteria, thus the possibility of apoptosis in hepatocyte-induced cells by KPC will be higher when compared with those that are *E. coli* ESBL infected.

## 1 INTRODUCTION

Sepsis is a clinical syndrome that occurs due to excessive body response to stimulation of microorganism products (Guntur, 2007). Sepsis is the leading cause of death in the world and the cause of deaths in Intensive Care Units (ICU). It is estimated that about 1,400 patients die in the ICU because of sepsis (Poeze et al., 2004). Apoptosis is commonly involved in bacterial infections and pathogenesis. During bacterial infections, virulent factors (mostly endotoxins) are produced and secreted from pathogens and trigger apoptotic signals. Research on caspase-3 is important, as it is the most important caspase effect responsible for morphology and biological changes seen in apoptotic cells (Ghatage et al., 2013). Based on this phenomenon, it is necessary to conduct research to

determine the increase of expression caspase-3 on the spleens and livers of rats infected with *Escherichia coli* Extended Spectrum  $\beta$ -lactamase (ESBL) and *Klebsiella pneumoniae* carbapenemase (KPC).

## 2 METHODS

### 2.1 Type and Design of Research

The type of this research is pure laboratory (true experimental) research using post-test only for the control group (data retrieval done after treatment) and compared with the control group.

## 2.2 Place and Time of Research

### 2.2.1 Research Place

This study was conducted in several locations: the Animal Unit Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga, Microbiology Laboratory of RSUD Dr. Soetomo, Surabaya, and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga.

### 2.2.2 Research Time

This study was conducted for approximately three months, from October 2017 to December 2017.

## 2.3 Research Objects

The object of the research used in this research was rats (*Rattus norvegicus*); a male strain *Wistar* aged about eight to 12 weeks with a body weight of 150–200 grams that came from the animal unit’s biochemistry laboratory, Faculty of Medicine, Universitas Airlangga.

## 2.4 Animal Treatment

Adapted rats were injected in the peritoneum section with the following treatments: 1) group one as normal control, i.e. injected aqua pro-injection-free pyrogen; 2) group two as treatment one, i.e. injected rat *E. coli* ESBL with dose  $1 \times 10^5$  CFU/ml; and 3) group three as treatment two, i.e. rat injected KPC with dose  $1 \times 10^5$  CFU/ml. After 24 hours post-exposure of polymicrobial sepsis animals will show apoptosis in the spleen and liver.

### 2.4.1 Caspase-3 Expression Observation on the Spleen and Liver of Rats

Observations of the caspase-3 expression on the liver and spleen of rats were performed by painting a primary antibody caspase-3 p12 subunit antibody (host: rabbit, target protein: caspase-3 p12 subunit, clonality: polyclonal, isotype: IgG, entrez gene: 836, source: KLH conjugated synthetic peptide derived from human caspase-3 p12 subunit, purification: purified by protein A) Bioss Antibodies production. The caspase-3 expression was observed using the immunohistochemical method. Caspase-3 expressed when exposed to the brown color in cytoplasmic sections, but if in clear cytoplasmic sections, it can be stated that caspase-3 is unexpressed. The

calculation of caspase-3 expression is done by calculating the cell expressing caspase-3 divided by all the preserved cells, then multiplying by 100%, so the data is expressed using a percentage.

## 3 RESULT

### 3.1 Caspase-3 Expression in Spleen of Rats with *E. coli* ESBL and KPC

Table 1 : Average Data and Standard Deviation Caspase-3 Expression in a Rat’s Spleen with Control Treatment, *E. coli* ESBL Infection, and KPC Infection (%).

| Group                     | x±SD            | Median | Max-Min |
|---------------------------|-----------------|--------|---------|
| Control (n=4)             | 4,25±0,5        | 4      | 5–4     |
| <i>E. coli</i> ESBL (n=4) | 33,75±3,86<br>2 | 33,5   | 38–30   |
| KPC (n=4)                 | 65,25±12,6<br>9 | 67     | 78–49   |

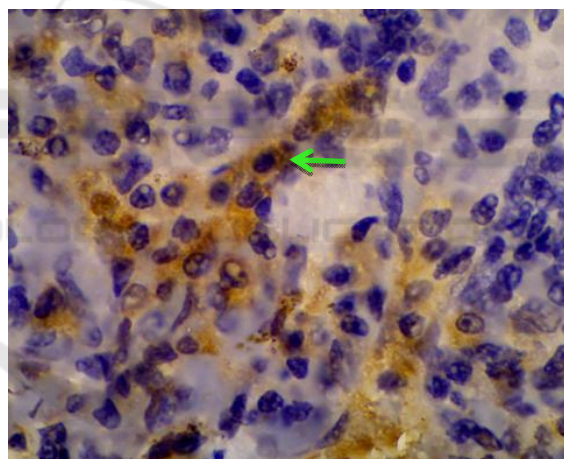


Figure 1 : Expression of caspase-3 in spleen of rats infected with *E. coli* ESBL are stained brown (green arrow). Magnification: x1000.

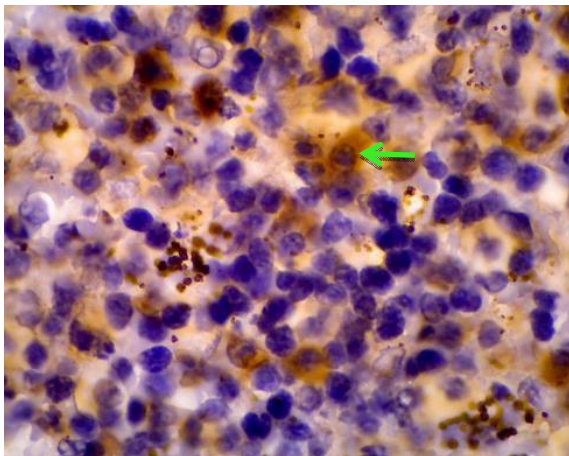


Figure 2 : Expression of caspase3 in spleen of rats infected with KPC are stained brown (green arrow). Magnification: x1000.

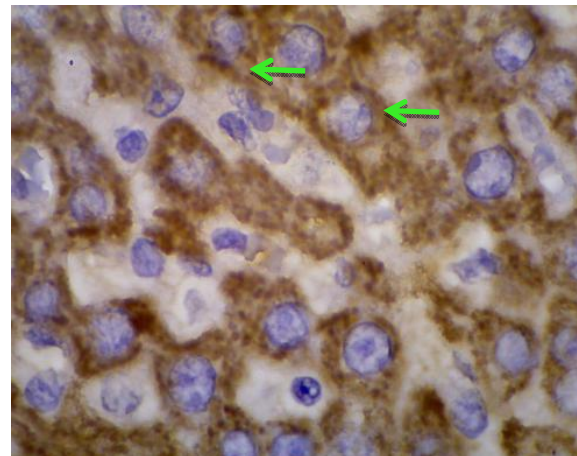


Figure 4: Expression of caspase-3 in the livers of rats infected with *K. pneumoniae* carbapenemase are stained brown (green arrow). Magnification: x1000.

### 3.2 Caspase-3 Expression in the Livers of Rats with *E. coli* ESBL and KPC

Table 3.2 Average Data and Standard Deviation Caspase 3 Expression on Rat's Liver with Control Treatment, *E. coli* ESBL Infection, and KPC Infection (%).

| Group                     | x±SD            | Median | Max-Min |
|---------------------------|-----------------|--------|---------|
| Control (n=4)             | 9,5±2,38        | 9,5    | 12-7    |
| <i>E. coli</i> ESBL (n=4) | 48,75±6,29<br>2 | 46,5   | 58-44   |
| KPC (n=4)                 | 58,75±4,03<br>1 | 59     | 63-54   |

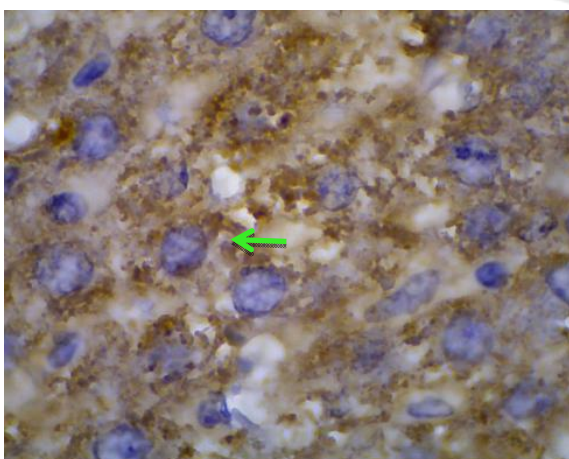


Figure 3 : Expression of caspase-3 in livers of rats infected with *Escherichia coli* ESBL are stained brown (green arrow). Magnification: x1000.

## 4 DISCUSSION

### 4.1 Caspase-3 Expression in the Spleen of Rats with *E. coli* ESBL and KPC

Increased caspase-3 expression in rat-infected KPC was higher when compared with those infected with *E. coli* ESBL. It can be influenced because of differences in antigens possessed by KPC with *E. coli* ESBL. The KPC capsule consists of an O antigen which is a liposaccharide consisting of a repeating polysaccharide unit. Antigen O is resistant to heat and alcohol. The second antigen is the K antigen. The K antigen is outside the antigen O and is a capsular polysaccharida. The K antigen may interfere with agglutination through antiserum O and is associated with virulence. Both of these antigens increase the pathogenity of KPC.

During bacterial infections, virulent factors are produced and secreted by pathogens and trigger apoptotic signals. In general, cells undergo apoptosis of two main pathways, extrinsic pathways (dead receptor pathways) and intrinsic pathways (mitochondrial pathways) (Jin and El-Deiry, 2005; Ayala et al., 2007). After releasing specific pro-apoptotic proteins, such as cytochrome c, smac/DIABLO, AIF, and Endo G, the execution path begins with caspase activation 3. Its main purpose is to bind and activate the caspase recruitment domain (CARD), Apaf-1, and procaspase 9, which leads to the formation of apoptosome. Next, it leads to activation of caspase-9 and further

activates the caspase-3 effector, which completes the apoptotic pathway. These are apoptosome formations and activation of the caspase effectors that cause apoptotic events, such as chromatin condensation, plasma membrane asymmetry, and cellular blebbing (Abud, 2004; Nikitakis et al., 2004).

## 4.2 Caspase 3 Expression in the Livers of Rats with *E. coli* ESBL and KPC

Based on previous research, it was found that there was an increase of caspase-3 expression in the livers of the rats in the group infected with *E. coli* ESBL and infected by KPC. Injection of bacteria in the rats was done through an intraperitoneal injection pathway. Bacteria injected into the peritoneum cavity will be absorbed into the portal circulation and transported to the liver. As an organ that acts as a recipient of portal and arterial blood vessels, the liver is an important component in the defense against blood-borne infections.

Increased caspase-3 expression in rats infected with KPC was higher when compared with *E. coli* ESBL-infected rats. This might be affected by differences in soluble factors of bacteria that can induce host cells. Factors involved in the virulence of KPC strains include capsular serotypes, lipopolysaccharides, iron-scavenging systems, fimbrial and non-fimbrial adhesions. The polysaccharide capsule surrounding KPC protects itself against the action of phagocytosis and serum bactericidal and may be considered the most important determinant of the virulence of KPC.

Liver damage is associated with the incidence of liver cell apoptosis (Mordue et al., 2001). Apoptosis through intrinsic pathways in liver cells is caused by a soluble factor of bacteria that can induce host cells, and is thus toxic to other cells. This soluble factor causes the mitochondria to release ROS. These bacterial infections cause the mitochondria to produce ROS and trigger the release of cytochrome *c* (Nomura et al., 2000). Cytochrome *c* will trigger caspase-9 to bind to the caspase effect, i.e. caspase-3, resulting in apoptosis (Yoon et al., 2002).

## 5 CONCLUSIONS

The increase in the caspase-3 expression in the spleens of rats infected with KPC was higher

compared with that of rats infected with *E. coli* ESBL. The different antigens in those two different bacteria may have contributed to the expression of caspase-3 and the possibility of apoptosis in the lymphocyte cells caused by KPC would be higher when compared with those infected with *E. coli* ESBL. Similarly, the different expression of caspase-3 in the liver of rats, infected with those two bacteria, may be caused by the soluble factors secreted by both bacteria.

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