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Wistar Rat Pneumonia Model: Bacterial Dose, Incubation Time, Inoculation Technique and Laboratory Examination Parameters

Ana Majdawati[1,5], Reviono[1,2], Hartono[1,3], Nestri Handayani[4], Soetrisno[1]

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ABSTRACT

Background: Making animal models is important before research involving human diseases. This study aimed to prepare animal models of pneumonia through the intranasal technique with a focus on the effect of the dose of *Klebsiella pneumoniae* (*Kp*) and incubation period on diagnosing pneumonia. We use male Wistar rats 6-8 weeks, 200-300 grams as much as 64 that appropriate the inclusion and exclusion criteria.

Methods: The research method was the pre-posttest group design to prove the creation of a Wistar Rat pneumonia model based on the dose and incubation time for diagnosing Pneumonia. There were On days 1 to 5, each of the 4 rats underwent a thoracic radiography (CXR) 64 rats model divided into 4 groups, N: 4 healthy; T1, T2 and T3 groups of rats were inoculated with 25, 50 and 100 µL of 0.5 McFarland intranasal *Kp* solution. A group of rats was then sacrificed, followed by retrobulbar blood collection (leukocyte-neutrophil count) and bacterial count/NaCL and lung tissue was taken histopathology and bacterial count/Brain Heart Infusion (BHI). The independent variables were the dose of *Klebsiella pneumoniae* (*Kp*) and the incubation period. The statistical analysis of the correlation of dose of bacteria, incubation time with leucocyte and neutrophils used One Way Anova, Kruskal Wallis if the data were abnormally distributed. The correlation is the dose of *Kp*, incubation period with chest radiography, histopathology, blood bacterial count (BBC) and pulmonary bacterial count (PBC) using the Chi-square test.

Result: The results showed that the dose of *Kp* was positively and significantly correlated with the neutrophil count (p=0.030) and the histopathology (p=0.000). Similarly, the incubation period was positively and significantly correlated with chest radiography (p=0.000) and histopathology (p=0.013).

Key words: Dose of *Klebsiella pneumoniaee*, Incubation time, Intranasal technique, Rats model pneumoniae.

INTRODUCTION

Animal models are living animals that are often genetically engineered and are used to investigate human diseases so that human beings are not directly harmed. Biological activity in animal models does not guarantee the expected effects on humans, although many medicines, treatments and healing of human diseases are developed with the guidance of animal models (Kari *et al*., 2007). Previous research and theories have mentioned that animal models should not suffer death or disability because of the treatment. The animal models must be appropriate to a specific disease that has already been proven by valid examination assessment (Kohn and Clifford, 2002). Additionally, it is crucial to consider the Biological Safety Levels (BSL) of both the laboratory where the test animal is housed and the research facility (Madenspacher and Fessler, 2016). The highest Community Acquired Pneumonia (CAP) causing bacterial pneumonia in Indonesia is *Klebsiella pneumoniae* (Sutanegara *et al*., 2019). The prevalence of the incidence of Multi-Drug Resistance (MDR) *Klebsiella pneumoniae* (*Kp*) in 2017 was 31.15% and increased in 2018 by 31.17%.

Based on the explanations given above, this study aimed to examine the effect of the dose of *Klebsiella pneumonia*e bacterial solution and incubation period on the pneumonia diagnosis in the development of animal models of pneumonia using Wistar rats (*Rattus norvegicus*) through the intranasal technique (Ekici *et al*., 2020). This study was new and has not been reported by other authors yet. The novelty of this research will be used as an essential reference for animal modelling of cases of Pneumonia in general and Pneumonia caused by *Klebsiella pneumoniae*. The study might help researchers to determine the dosage of bacteria and the time required to choose specimens based on the parameters to be tested.

[1]Doctoral Program of Medical Science, Universitas Sebelas Maret, Surakarta, Indonesia.
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia.
[3]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia.
[4]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia.
[5]Department of Radiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia.

Corresponding Author: Ana Majdawati, Doctoral Program of Medical Science, Universitas Sebelas Maret, Surakarta, Indonesia. Email: ana.majdawati@umy.ac.id

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MATERIALS AND METHODS

This research was conducted under the ethics committee of Laboratorium of Penelitian dan Pengujian Terpadu (LPPT) UGM with the Ethical Clearance certificate number of 000023/04/LPPT/ IX/2021. This study used a pre-post-test control group design to compare various doses of *Klebsiella pneumoniae* solution with the incubation times of pneumonia in Wistar Rats pneumonia models. The independent variables were doses of 25 µL, 50 µL and 100 µL of the 0.5 McFarland standard bacterial solution and the incubation times of 1-5 days. The parameters used as diagnostic references for pneumonia (dependent variables) were leukocyte and neutrophil numbers, thoracic radiography, bacterial count in lung tissue and blood and histopathological examination of lung tissue. The sample examinations, were carried out by 2 experts in their fields, are not related to each other and are double-blind. The Kappa value of the examiner must meet the requirements, namely 80-100%.

The 64 rats were used in the study based on the inclusion and exclusion criteria.

Inclusion criteria: Male Wistar rats, healthy and not physically disabled (have a health certificate from the institution or agency that provides the test animals), age 6-8 weeks, weight 200-300 grams and previously have never been studied.

Exclusion criteria: Illness/infection during the adaptation period, death during treatment.

The minimum sample size was calculated based on the Federer formula (Federer, 1955):

$$(t - 1)(n - 1) > 15$$

t = Number of groups.
n = Number of subjects per group.

In this study the number of groups = 16, n = The number of samples per group.

Based on this formula, the number of samples of test animals was >2 [(16-1) (n-1) > 15]. So, the planned number of samples per group was 4 Wistar Rats. The required number of test animals was 16×4 = 64 Wistar Rats (Muhammad, 2016).

Preparation of a solution of *Klebsiella pneumoniae* bacteria

Isolates of *Klebsiella pneumoniae* were sub-cultured on McConkey Media and a bacterial solution was made according to 0.5 McFarland standards. The procedures included; take several bacterial colonies from 24-hour growth on Mc Conkey media, then suspend it into 0.5 mL on liquid Brain Heart Infusion (BHI) media, incubate for 5-8 hours at a temperature of 37°C, then add the bacterial suspension to sterile aquadest up to certain turbidity under 0.5 McFarland standard equivalent to germ concentration of 1.5×10^8 CFU/ml (CFU = Colony Forming Unit) (Gayathiri, 2018).

Making model rats and treatment of Wistar Rats

All the 64 male Wistar rats were acclimatized for two weeks before treatment. The preservation of animals was in the test animal laboratory of PAU-UGM, meeting BSL-2 standards by regulating room conditions, temperature, air humidity and air circulation.

The 64 Wistar rats were divided into four groups with Group II, III, IV having 20 rats each and Group I had 4 rats. Group I was not infected with *Klebsiella pneumoniae* and was kept as test control. Group II, III and IV, were infected with doses of the 0.5 McFarland standard bacterial solution of 25, 50 and 100 µL, respectively. On day 1, Wistar Rats were inoculated with *Klebsiella pneumoniae* solution from isolates of pneumonia patients at Yogyakarta City Hospital. The intranasal inoculation method with micropipettes measurement 1-100 µL, 0.5 McFarland standard was done by positioning the head and nose of the rats facing upwards (Fig 1). In this study, we used the intranasal technique with a blunt tip micropipette because it was easy to get and obviously did not injure the rats.

Laboratory examination of the parameters

In each group, at each incubation period, blood samples of 4 rats were taken from the retroorbital plexus using a hematocrit pipette of 1 mL, in which a 0.5 mL was placed in a sterile liquid BHI tube for bacterial count examination and a 0.5 mL was placed in the EDTA tube for examination of the leukocytes and neutrophils' count.

The animal models with pneumonia were then subjected to thoracic radiography at the Animal Hospital, Fakultas Kedokteran Hewan, Universitas Gadjah Mada in a ventrodorsal position. taking chest radiography, the animal models were anaesthetized using from a mixture of ketamine and Xylazine dengan komposisi 70 mg/kg weight body and Xylazine 6 mg/kg weight body, intramuscularly or intraperitoneal (Mustika Anggiane Putri, n.d.). Xylazine is a structural analog of Clonidin and an alpha-2 adrenergic receptor agonist. Xylazin is a common veterinary drug used for sedation, anesthesia, muscle relaxation and analgesia in animals. It is often in combination with Ketamine (Ruiz-Colón *et al.*, 2014).

Rats' model pneumonia consisted of 4 groups (N,T1, T2 and T3) all underwent chest radiographic with anesthesia, than continued sacrificed. Rats' model underwent chest

Fig 1: Inoculation technique of *Klebsiella pneumoniae* solution (intranasal).

surgery and pulmonary retrieval was performed by cutting the trachea to the bronchus to obtain Bronchoalveolar Lavage (BAL) fluid. The fluid obtained was placed in a sterile NaCl tube for germ number examination. Histopathological examination was conducted by taking intact lung tissue and putting it in a potio containing 10% formalin until all lung tissue was immersed in formalin. The histological sections of the lung tissue of the animal models were prepared using Hematoxylin - Eosin and were examined under a microscope at a magnification of 40X.

The count of bacteria

The examination of the isolated subculture of *Klebsiella pneumoniae* and bacterial count was done at the Clinical Microbiology Laboratory of the Public Hospital of Asri Medical Center, Yogyakarta. Examination of bacterial count used blood samples and trachea-bronchus tissue. The bacteria were cultured on a selective McConkey media with dilution, scratched using the quadrant streak plate method with "the ose" (a loop rod or loop wire is a tool used for bacterial inoculation in petri dish) (Thresia *et al.*, 2021). Inoculation is the process of planting bacteria using agar media in a petri dish. having a size of 2/1000 and then incubated for 24 hours. The bacterial count were calculated using Equation by Gayathiri, 2018:

$$\text{Bacterial counts (BC)} = X \times DF \times 500 \text{ CFU/cm}^2$$

Where:
BC: Sum of bacterial count in CFU/cm^2.
X: Number of colonies counted in McConkey media.
DF: Dilution factor.
500 CFU: Obtained from 1 mL/small ose volume of 0.002.

Thoracic radiography

Radiological examination was carried out at the veterinary teaching hospital (RSHP UGM). Radiology imaging of the rats lung, the rats were anaesthetised before being placed on a radiographic sensor (Krystal X easy digital, Owandy software) at a distance of 80 cm from the X-ray source.25,26 thoracic radiography assessment was done by using an X-ray machine (Intra Scan DC Skan Ray, with IV- 1 phase 230 V, IF- 50 Hz, Momentary and with a 48 kVp exposure for 0.5 mAs. The assessment of pulmonary lesion degrees was divided based on the extent of the lesions, score 0: Normal (no lesions in both lungs), score 1: mild degree (minimal lesions on one lung dextra or sinistra); Score 2: Medium-Severe Degree (lesions regarding two pulmonary fields of right and left) (Fouriez-Lablée *et al.*, 2017).

Histopathology

Hematoxylin-Eosin (HE) painting was conducted at the Pathology Anatomy (PA) Laboratory of the Public Hospital of Asri Medical Center, Yogyakarta. The assessment of the microscopic examination of pulmonary preparations results was calculated from the number of scores of each variable (edema, neutrophilic infiltration, inflammatory interstitial and congestion). The scores ranged from 0 (normal) to 3 (severe), in which the average values were recorded as semi-quantitative histological indicators of lung injury (Gui *et al.*, 2021; Mikerov *et al.*, 2011). The score 1: Mild: signs of hemorrhagic inflammation consisting of neutrophils, erythrocytes, fibrin and necrotic cellular debris rare/field viewing; score 2. moderate: signs of hemorrhagic inflammation consisting of neutrophils, erythrocytes, fibrin and necrotic cellular debris more tightly / field viewing; score 3. Severe: hemorrhagic inflammatory signs consisting of neutrophils, erythrocytes, fibrin and necrotic cellular debris tight / field viewing (Meyerholz *et al.*, 2018).

Leucocytes and neutrophils

Leukocytes and neutrophils were examined in the Pathology Clinic Laboratory of the Public Hospital of Asri Medical Center, Yogyakarta. Routine blood tests were done by making blood smears and counting the number of leukocytes and neutrophils.

Statistical analysis

The statistical analysis of One-Way ANOVA was used to examine the correlation between the dose of the *Klebsiella pneumoniae* solution and the incubation period with leucocyte and neutrophil. Kruskal-Wallis test was used when the data was in abnormal distribution. Chi-square test was used for analytic correlation of the dose of the *Klebsiella pneumoniae* solution and the incubation period with thoracic radiography results (categorical data), bacterial counts (categorical data) and histopathology (categorical data). The statistical analysis was conducted using the IBM SPSS Statistics for Windows Release 25 program. The test results were significant when the *p*-value was < 0.05 (Muhammad Sopiyudin Dahlan, 2016).

RESULTS AND DISCUSSION

The Wistar Rat (Rattus Norvegicus) has a higher potential as an animal model of pneumonia than the others, which were used in previous studies (Meyerholz *et al.*, 2018), because of its larger body and lungs, so that chest radiographic examination is easier. Therefore, this study used Wistar rats as animal models (Metersky and Waterer, 2020).

Bacterial inoculation techniques for animal models are primarily intratracheal/oropharyngeal (Bergamini *et al.*, 2021) or intranasal (Mizgerd and Skerrett, 2008) or intravenous technique (Widjiati, 2021) with anaesthesia. This study used intranasal techniques as the endotracheal tube (ET) and laryngoscope used for intratracheal inoculation were unavailable and this technique is more natural. The intranasal inoculation in the present study was carried out without anesthesia.

The result of the statistical analysis is shown in Table 1. The correlation between the dose of *Klebsiella pneumoniae* solution and incubation period with the number of neutrophils, correlation between the dose of *Klebsiella pneumoniae* solution and incubation period with

Table 1: Pneumonia diagnosis results in Wistar rats based on leukocyte number, neutrophils, bacterial cultures from pulmonary and blood preparations, pulmonary histopathology results and thoracic radiography.

Diagnosis parameters	Positive results	Information	*p-value*
Leukocyte-*Kp* dose and incubation period	Day 1	Dose 25, 50, 100 µL	0.520
Neutrophils-*Kp* dose and incubation period	Day 1 drop, day 1,3,4 and increases sharply until day 5 (dose 50 µL)	Dose 25, 50, 100 µL	0.030*
PBC-*Kp* dose	Day 1, 3 increase	Dose 50, 100 µL	0.760
PBC-Incubation period	Day 1, 3 increase	Dose 50, 100 µL	
BBC-*Kp* dose	Day 1, 2, 3, 5 increase	Dose 25, 50, 100 µL	0.593
BBC-Incubation period	Day 1, 2, 3, 5 increase	Dose 25, 50, 100 µL	0.428
Histopathology-Kp dose	Day 1, 2, 3, 4, 4	Dose 25, 50, 100 µL	0.000*
Histopathology-Incubation period	Day 1, 2, 3, 4, 4	Dose 25, 50, 100 µL	0.013*
CXR-Kp dose	Day 3, 4, 5 begin to see consolidation, or increased lung density	Dose 25, 50, 100 µL	0.996
CXR- incubation period	Day 3, 4, 5 begin to see consolidation, or increased lung density	Dose 25, 50, 100 µL	0.000*

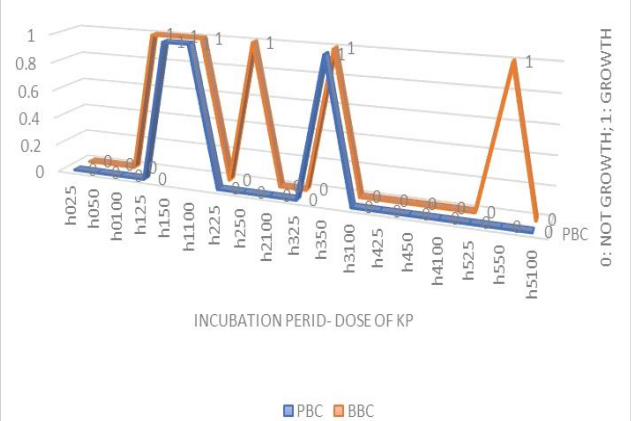
PBC- Pulmonal bacterial count; BBC- Blood bacterial count; CXR- Chest X Ray; *Kp- Klebsiella pneumonia;* *Significant at $p \leq 0.05$; NS- Non-significant at $p > 0.05$.

histopathology of lung tissue and the correlation between the incubation period of pneumonia with the chest radiography were significant. Pneumonia with the highest neutrophil number parameters occurred on day 5 at a dose of *Klebsiella pneumoniae* solution of 25 µL (neutrophils: 52.3%), on day 5 at a dose of *Klebsiella pneumoniae* solution of 50 µL (neutrophils: 61.6%) and on day 1 at a dose of *Klebsiella pneumoniae* solution of 100 µL (neutrophils: 46%). Normal values of neutrophils in Wistar Rats were in the range of 12-38% (Giknis and Clifford, 2008). After inoculating *Klebsiella pneumoniae*, neutrophil values increased on the first day (24 hours) and were commonly found in the air way or respiratory tract (Liu *et al.*, 2020; Madenspacher and Fessler, 2016). The dose of 100 µL, 0.5 McFarland standard on the first day (24 hours), neutrophil values in this group were high.

Previous studies reported leukocytes to increase at 24-48 hours post-inoculation and decrease after 72 hours post-inoculation (Dong *et al.*, 2012; Liu *et al.*, 2020; Madenspacher and Fessler, 2016). Overall, the results of this study showed that the number of leukocytes was high (more than normal, *i.e.* 2-10 × 10^3/mm^3) in post-inoculation because of the infection process. However, the results did not show a significant correlation between the dose of inoculated bacteria and post-inoculation time to the number of leukocytes (Chakotiya *et al.*, 2018). Statistical test results for the relationship between *Klebsiella pneumoniae* dose and pneumonia incubation time based on the neutrophil count were significant (p=0.030).

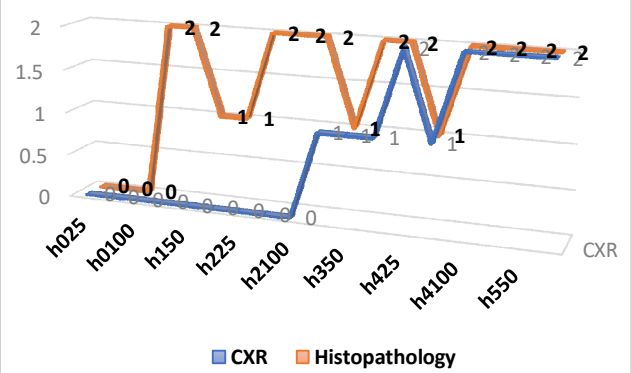
The relationship between *Klebsiella pneumoniae* (*Kp*) dose and incubation time was based on parameters of blood bacterial count (BBC) and pulmonal bacterial count (PBC) (Fig 2A) and based on chest radiography and histopathology (Fig 2B).

The highest degree of lesion on the thoracic radiography occurred on days 4 and 5 at the dose of *Klebsiella pneumoniae* solution of 25 µL (score 3), on days 3, 4 and 5



0 = No bactrial growth kp; 1 = Showing bacterial growth Kp.

Fig 2A: Graph of infection timing at *Klebsiella pneumoniae* solution doses and incubation periods with bacterial growth from the lung tissue and blood specimens.



CXR- 0: Normal; 1: Minimal lesions; 2: Moderate-severe lesions. Histopathology- 0: No inflammation; 1: Mild inflammation; 2: Moderate severe inflammation.

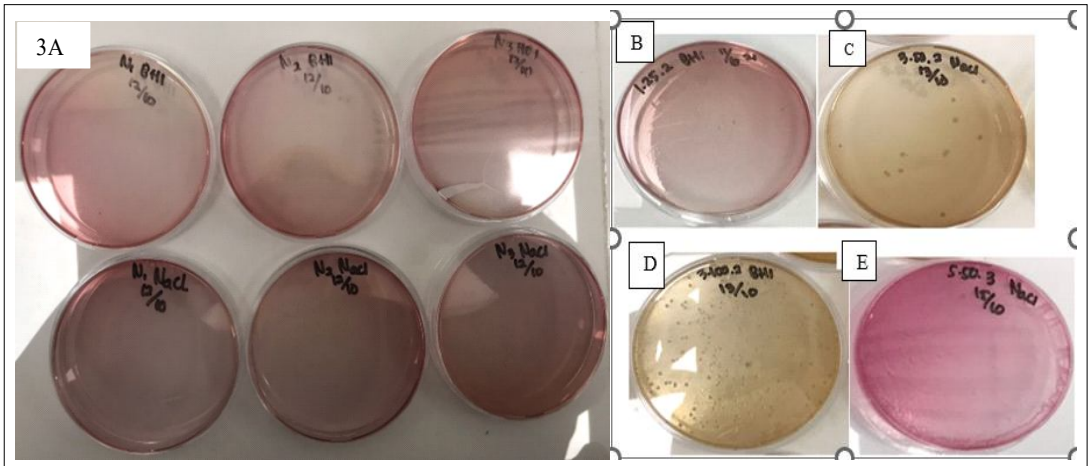
Fig 2B: Thoracic radiography graph based on the doses of *Klebsiella pneumoniae* and the time of infection.

at the dose of *Klebsiella pneumoniae* solution of 50 µL (score 3) and on days 4 and 5 at the dose of *Klebsiella pneumoniae* solution of 100 µL (score 3). The results of this study showed that chest radiography score had a significant correlation with the length of incubation time, which was about 3-5 days with a dose of *Klebsiella pneumoniae* 25, 50 and 100 µL, 0.5 McFarland standard (Fig 2B). Pneumonia in the pulmonary parenchyma was caused by an inflammatory process due to *Klebsiella pneumoniae*. The body's defense factors can cause these bacteria to die, triggering macrophage alveolars, oedema fluid, polymorphonuclear cells (neutrophils) and erythrocytes filling the alveoli and interstitials. This inflammatory process appeared on thoracic radiography as opacity or consolidation in the pulmonary field. The consolidated picture could be seen on thoracic radiograph after the initial inflammatory/acute. The extent of consolidation on chest radiograph was influenced by the dose of bacteria entering the lungs, the length of occurrence time of infection and the location of the lesion (Liu *et al*., 2020; Mizgerd and Skerrett, 2008). Chest radiograph is sometimes unable to show apparent lesions due to several reasons, the location of the lesions that are in the posterior or parts of the lungs that are super positioned with other parts of the lungs, or large lesions that are still minimal. A more accurate radiological examination with a higher diagnostic value is the chest Computerized Tomography Scanning (CT-scan), but its application to animals is still very limited (Fouriez-Lablée *et al*., 2017; Ogur *et al*., 2019).

The incidence of pneumonia with the parameter of the bacterial count in pulmonary preparations indicated the growth of gram-negative bacteria on days 1, 2, 3 and 5. Whereas in the blood specimen, the bacterial count showed growth on the first and third days. The results of statistical tests showed no significant correlation between the dose of bacterial solution and the incubation time with the bacterial count from lung tissue and blood specimens. Microorganisms (*Klebsiella pneumoniae*) can enter through the respiratory tract and get to the alveoli after 8 hours. This study showed positive culture results in both samples, namely from lung tissue and blood on day 1 at a dose of 25 µL, 0.5 McFarland standard (Fig 2A,3). The bacteria can persist for a long time in the lung tissue and can be found earlier in the first 24 hours in the lung tissue of the wistar Rat intranasally. Intravenous administered bacterial solution inoculation caused bacteria to be found in the blood after the first 24 hours of incubation. In the lung tissue, the bacteria were found after 48 hours and will be systemically circulated into blood vessels (bacteriemia) (Madenspacher and Fessler, 2016). Fig 3A-E shows the culture results of blood and lung specimens on McConkey's selective media showing the growth of *Klebsiella pneumoniae* colonies.

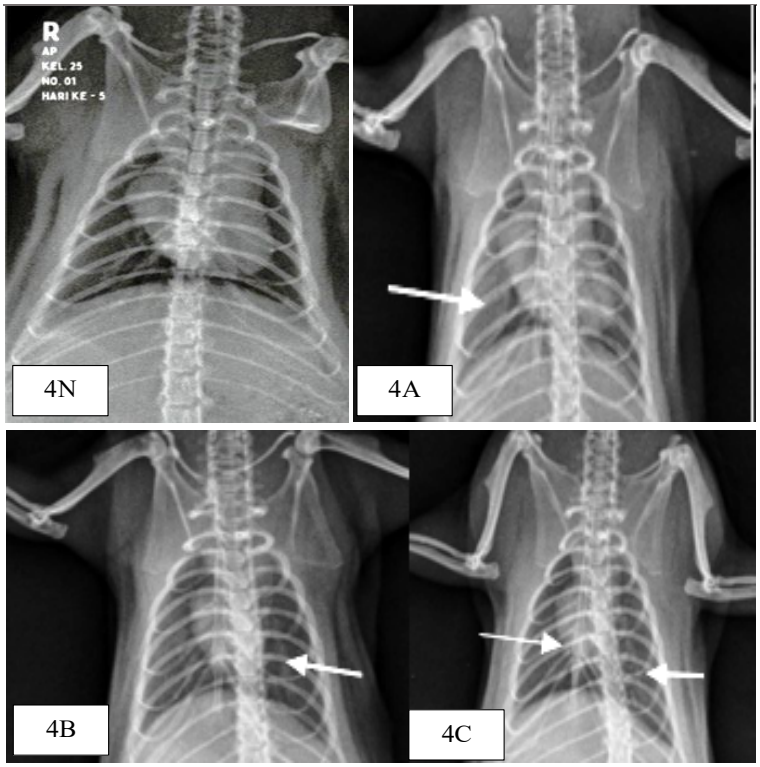
Fig 4 (N, A-C) shows the results of chest radiography from Wistar Rats with a pneumonia model with an appearance of the lungs without lesions (N) and an appearance of opacity lesions indicating an inflammatory reaction (Pneumonia).

Histopathological examination Inflammatory degree on the first day (day 1) already indicated the presence of mild and moderate degree inflammations. Severe inflammatory degree occurred on day 1 (dose of *Klebsiella pneumoniae* solution of 100 µL), day 2 (dose of *Klebsiella pneumoniae* solution of 50 µL) and days 4-5 (dose of *Klebsiella pneumoniae*



3A) Results of blood cilture and lung tissue from Wistar Rat with normal control (Results: no bacterial groeth on McConkey media); 3B) Pulmonary culture results (BHI) on day 1 with a dose of *Klebsiella pneumoniae* solution 25 µl, 0.5 McFarland standard showed the growth of a colony of *Klebsiella pneumoniae* of small/minimal amount; 3C) Blood culture (NaCl) on day 3 with a dose of *Klebsiella pneumoniae* solution 50 µL, 0.5 McFarland standard, small/minimal amount; 3D) Pulmonary culture results (BHI) on day 3 with a dose of *Klebsiella pneumoniae* solution 100 µL, 0.5 McFarland standard showed the growth of colonies of large amount of *Klebsiella pneumoniae* bacteria; 3E) The results of blood culture (NaCl) on day 3 with a dose of *Klebsiella pneumoniae* solution 50 µL, 0.5 McFarland standard showed the growth of colony of moderate amount of *Klebsiella pneumoniae*.

Fig 3: Blood and Pulmonary culture results in the group of mice were normal and post-inoculation on the first day until the fifth day.



Normal Wistar Rats thoracic radiograph model (4N), Wistar rats modeled pneumonia on day 3 showing lesions in the right lung fit to mild pneumonia (4A), Wistar rats model pneumonia on day 4 showing lesions in the left lung fit to mild pneumonia (4B), Wistar rats modeled pneumonia on day 3 showing lesions in the right and left lungs fit to severe pneumonia (4C).

Fig 4: Chest X Ray in normal and pneumonic Wistar rats.

solution of 50-100 µL). Some histopathological parameters indicated an inflammatory degree based on the number of polymorphonuclear cells (leukocytes, neutrophils, erythrocytes/bleeding), vascular thickening, bronchus wall and the alveoli areas thickened (epithelial layer of the alveoli) (Ogur *et al.*, 2019).

Data from the study showed that the number of leukocytes and neutrophils in blood samples, which were parameters of pneumonia in Wistar Rats, showed high results from day 1 at all doses of bacterial solution. An increase in leukocytes and neutrophils number are reported (Dong *et al.*, 2012) at 24 hours and 48 hours after inoculation of *Klebsiella pneumoniae* in animal models (Table 1). Systemically, the leukocytes and neutrophils numbers in the inflammatory process of acute pneumonia can be judged from peripheral blood preparations. Pro-inflammatory cytokines and Chemocin (IL-1β, IL-6, IL-12, TNF-α) showed improvement on hours 24 and 48 (Madenspacher and Fessler, 2016).

Histopathological examination showed inflammation with an average score of 2 on day 1 post-inoculation. It occurred at all doses of the bacterial solution of *Klebsiella pneumoniae*. Histopathology of the pulmonary parenchyma indicated alveolar inflammation and an increase in the number of intra-alveolar neutrophils on days 1,2,3 (Di Bonaventura *et al.*, 2010). The bacterial count in the blood and lung tissue cannot be used as a parameter because it gives invalid or inconsistent results. Based on these data, researchers concluded that pneumonia in model animals occurred on day 3 at doses of 25, 50 and 100 µL, 0.5 McFarland standard. Pneumonia diagnosis based on the number of leukocytes, neutrophils and histopathology of the lung tissue occurred since the first day of post-inoculation, but visually the density of the lung tissue increased in chest radiography only seen on day 3. This study showed that lung density increased on day 3 post-bacterial inoculation. Chest radiography showed abnormal results with an increase in pulmonary density occurring from day 3 at all doses of *Klebsiella pneumoniae* solution. Inflammatory parameters in the radiographic picture (Fig 4 A-C) indicated improvement in the density of the pulmonary parenchyma, indicating inflammation of the alveoli. The fluid filled the lumen alveoli, transudate, or exudate (Meyerholz *et al.*, 2018).

The parameters used for the successful development of pneumonia animal models can be inferred from the explanation in Table 1. The data presented showed a significant correlation between: doses of 25, 50 and 100 µL, 0.5 McFarland standard on day 1 post-inoculation with an increase in number of neutrophils (*p*: 0.030); doses of 25, 50,

100 µL, 0.5 Mc Farland standard on days 1 to 4 post-inoculation with pulmonary histopathological parameters (HE) (*p*: 0.00) and (*p*: 0.013); doses of 25, 50 and 100 µL, 0.5 McFarland standard on days 3 to 5 post-inoculation with thoracic radiography screening parameters (*p*: 0.000) (Table 1).

Some factors need to be considered in the development of animal models. They are the dose of inoculated bacteria, the length of incubation time, the host's immune response factor, the virulence level (hypervirulent) of microorganisms (*Klebsiella pneumoniae*), rat homogenization (strain uniformity, weight, sex, feeding, rat rearing site), microorganism inoculation techniques (*Klebsiella pneumoniae* and others) (Bengoechea and Sa Pessoa, 2019; Joseph *et al.*, 2021; López Hernández *et al.*, 2015).

CONCLUSION

In this study, animal models of Pneumonia from Wistar Rats, are intranasally inoculated were not anaesthetized with *Klebsiella pneumonia*e solution doses of 25, 50 and 100 µL, 0.5 McFarland standard. There were unequal results in the Pneumonia diagnosis, depending on the parameters used. Based on the results of this study, it can be concluded that several parameters that influence the diagnosis of pneumonia in Wistar Rats are as follows: the dose of *Klebsiella pneumoniae* solution for the diagnosis of pneumonia has a significant influence on the neutrophil number (*p:* 0.030) and the incubation time for the diagnosis of pneumonia has an influence significant effect on thoracic radiography results (*p:* 0.000). Based on the *Kp* dose and incubation time, the histopathology results had a significant effect on the diagnosis of pneumoni a (*p:* 0.000 and p: 0.013). The dose of *Kp* solution and incubation time did not have a significant effect on the diagnosis of pneumonia in Wistar rats based on PBC germ numbers (p: 0.760; p: 0.428) and BBC (p: 0.93; p: 0.535).

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Conflict of interest

All authors declared that there is no conflict of interest.

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