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# Identification of bacteria from sputum specimens of pneumonia patients based on age and gender at Jenderal Ahmad Yani regional hospital, metro city

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## Abstract

Pneumonia is an airborne infectious disease that poses a serious global health threat. One of the high-risk groups for pneumonia includes infants and the elderly aged 65 years and above. In elderly patients, pneumonia is often associated with severe complications and can lead to death. Although various efforts have been made to prevent and treat pneumonia, the infection rate remains high. This study aims to identify the types of bacteria found in sputum specimens from pneumonia patients based on age and gender at RSUD Jenderal Ahmad Yani Kota Metro. The research is descriptive in nature and uses a survey method based on available data and specimens from the hospital. The data obtained from bacterial identification were analyzed descriptively and presented in tables and figures. The results showed that out of 22 sputum specimens, both Gram-positive and Gram-negative bacteria were found. Ten specimens tested positive for Gram-positive bacteria, while twelve showed Gram-negative bacteria. The most frequently identified bacterial species were *Klebsiella pneumoniae, Staphylococcus aureus, and Staphylococcus epidermidis*. *Klebsiella pneumoniae* was predominantly found in female patients, while *Staphylococcus aureus* was more common in male patients. Patients aged over 60 were more susceptible to Gram-negative bacterial infections. These findings provide initial insights into the bacterial profiles associated with pneumonia and are expected to support more targeted prevention and treatment strategies.

**Keywords:** *Pneumonia; Sputum*; Gram-Positive Bacteria; Gram-Negative Bacteria; *Klebsiella pneumoniae*; *Staphylococcus aureus*; Age; Gender

## 1. Introduction

Pneumonia is an airborne infectious disease, making it a global health concern that requires serious attention. One of the high-risk groups for pneumonia includes young children and the elderly, especially those aged 65 years and above [1]. Pneumonia, commonly referred to as "wet lungs," is a condition in which an individual experiences an infection in the air sacs of the lungs. This infection can affect either one or both lungs. Pneumonia is one of the leading causes of death among children worldwide. The World Health Organization (WHO) estimates that 15% of deaths in children under the age of five are caused by this disease. Furthermore, the WHO reported that in 2017, over 800,000 children died due to pneumonia [2].

Respiratory tract infections among children under five in Indonesia ranged from 20–30% between 2010 and 2014, with an increase in prevalence reaching 35.5% from 2015 to 2019. However, in 2020, the coverage slightly declined to 34.8%. The province with the highest prevalence of respiratory tract infections in children under five was DKI Jakarta at 53.0%, while Lampung Province ranked sixth, with a prevalence rate of 39.8%. According to medical records from RSUD Jenderal Ahmad Yani Kota Metro in the Pediatric Ward, respiratory tract infections ranked second among the top ten diseases in children, with pneumonia cases recorded in 214 children, accounting for 18.90% of cases. Severe respiratory

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tract infection symptoms in children include bluish lips and skin, nasal flaring during breathing, altered consciousness or unconsciousness, noisy (stridorous) breathing, signs of restlessness, rapid pulse rate exceeding 60 beats per minute, and a reddened throat [3]

The onset of symptoms such as fever and chills in pneumonia patients is a natural response to infection, indicating inflammation within the body. This prompts the hypothalamus to increase body temperature as a defense mechanism against invading pathogens. Other common symptoms include nausea and loss of appetite, which often result from excessive mucus production and persistent coughing. This productive cough increases pressure in the abdominal cavity and stimulates the central nervous system, contributing to digestive discomfort. Coughing itself serves as a reflex to expel irritants whether microbial or non-microbial from the respiratory tract, but in pneumonia, it reflects deeper infection reaching the bronchi and alveoli. Additionally, shortness of breath is frequently experienced due to mucus buildup that obstructs airflow, impairs gas exchange in the lungs, and leads to difficulty in breathing. If not addressed, these symptoms can escalate and negatively impact the patient's overall oxygenation and clinical condition[4].

Numerous strategies have been implemented to combat pneumonia, encompassing both preventive and therapeutic approaches. Preventive measures include minimizing exposure to risk factors such as tobacco smoke, air pollution, and crowded environments that facilitate transmission, avoiding direct contact with infected individuals, and promoting exclusive breastfeeding in infants as a form of passive immunity. Therapeutically, early medical intervention is essential when symptoms such as fever, cough, nasal congestion, hoarseness, dyspnea, or intercostal retractions are observed [5]. Pneumonia continues to be a significant global health burden, contributing to high morbidity and mortality rates, particularly among vulnerable populations. Common bacterial pathogens associated with pneumonia include *Klebsiella pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and Mycoplasma pneumoniae*. Despite advancements in prevention and treatment, the incidence of pneumonia remains substantial. Accordingly, this study aimed to identify the bacterial etiology of pneumonia in patients at RSUD Jenderal Ahmad Yani, Kota Metro, with a focus on stratification by age and gender to better understand the epidemiological distribution of the infection.

## 2. Material and methods

### 2.1. Study Location

This study was conducted between December 2024 and January 2025 at the Clinical Pathology Microbiology Laboratory of Jenderal Ahmad Yani Regional Hospital, Metro City.

### 2.2. Procurement of ingredients

The equipment used in this study included an analytical balance, beaker glass, Erlenmeyer flask, measuring cylinder, electric stove, stirring rod, Petri dishes, test tubes, test tube rack, paper, funnel, autoclave, inoculating loop, Bunsen burner, incubator, glass slides, microscope, Negative Combo 67 (NC 67) panel, Positive Combo 34 (PC 34) panel, panel holder, Laminar Air Flow cabinet, Prompt Inoculation System needle, panel wells, water bath incubator, computer, and microscan system. The materials utilized comprised Brain Heart Infusion (BHI) media, Thioglycolate (THIO) media, blood agar, MacConkey agar, blood, distilled water, sputum specimens, immersion oil, Gram stain reagents (crystal violet, iodine solution, acetone/alcohol 95%, safranin or carbol fuchsin), water, and tissue paper.

### 2.3. Procedure for Media Preparation and Specimen Inoculation

Sputum collection began with patient instruction, including mouth rinsing and removal of dentures if necessary. Patients were positioned standing or leaning forward when seated, and a 3–5 ml sample of sputum from the lower respiratory tract was collected in a sterile container, sealed, labeled, and sent to the laboratory for further analysis. Upon arrival at the laboratory, preparation of culture media was carried out by weighing BHI, THIO, blood agar, and MacConkey media using an analytical balance 4 g/100 ml for blood agar, 5.15 g/100 ml for MacConkey, 3.7 g/100 ml for BHI, and 3.6 g/100 ml for THIO. Each medium was dissolved in 100 ml of distilled water, heated while stirred until boiling, and then allowed to cool slightly. BHI (5 ml) and THIO (15 ml) were transferred into test tubes and sterilized in an autoclave at 121°C and 1 ATM for 15 minutes. Once cooled to approximately 40 °C, MacConkey medium was poured into Petri dishes to a thickness of about 3 ml, and blood agar was prepared by adding 10 ml of blood into the medium, then poured into Petri dishes with the same thickness. All prepared media were cooled to room temperature and stored at 2–8 °C. Subsequently, inoculation was performed by transferring the prepared sputum specimen into BHI and THIO media using an inoculating loop, followed by incubation at 34–36 °C for 18–24 hours.

### 2.4. Procedure for Bacterial Streaking from BHI Media to Blood Agar and MacConkey Media

The procedure began by removing the blood agar and MacConkey media from the refrigerator and allowing them to reach room temperature. Bacteria were then taken from the BHI medium using an inoculating loop and streaked onto both the blood agar and MacConkey media in a T-shaped quadrant pattern. The inoculated media were subsequently incubated at 34–36 °C for 18–24 hours.

### 2.5. Procedure for Gram Staining and Gram Reading

The procedure began by taking bacterial colonies from either the blood agar or MacConkey media using an inoculating loop, and then placing them onto a glass slide. The sample was spread evenly to avoid it being too thick or thin, and then fixed by passing it over a Bunsen burner three times. Next, Gram staining was performed using Gram A, B, C, and D reagents. The slide was first stained with Gram A (crystal violet) for 1 minute, then rinsed with running water. It was then stained with Gram B (iodine solution) for 1 minute, followed by rinsing with water. Afterward, Gram C (acetone/95% alcohol) was applied for 30 seconds, and the slide was rinsed until the crystal violet stain disappeared. Finally, Gram D (safranin/carbol fuchsin) was applied for 1 minute, and the slide was rinsed again. After drying, the slide was observed under a microscope with one drop of immersion oil, using a 100x magnification. The results were recorded, with Gram-positive bacteria appearing blue and Gram-negative bacteria appearing red.

### 2.6. Bacterial Identification Procedure

The procedure began by taking bacterial colonies from either the blood agar or MacConkey media using a Prompt Inoculation System loop from a known Gram-positive or Gram-negative colony. The colony was then transferred into a water incubation bottle containing incubation fluid, homogenized, and left to stand for approximately 20 minutes. After this period, the bacterial suspension was poured into the wells of a panel. For Gram-negative bacteria, the suspension was transferred into the Negative Combo 67 panel, while Gram-positive bacteria were transferred into the Positive Combo 34 panel using a panel holder. The panels were then incubated at 34–36 °C for 18–24 hours.

### 2.7. Procedure for Panel Reading to Identify Bacterial Species

After incubating the Negative Combo 67/Positive Combo 34 panels for 18–24 hours at 34–36°C, the results were read using a computer and Microscan device. Patient data and specimen type were entered into the system. The panels were placed on the Microscan panel block, and the bacterial identification results were recorded as they appeared. The findings were then saved. Observations of the results were conducted for all treatments, taking into account the patient's age and gender. Finally, the results were further analyzed using the computer system.

### 2.8. Data Analysis

The data obtained from the bacterial identification of sputum specimens from pneumonia patients, based on the patients' age and gender at RSUD Jenderal Ahmad Yani Kota Metro, were analyzed descriptively. The results were then presented in the form of tables and figures for clearer interpretation and comparison.

## 3. Results and discussion

The purpose of this study was to evaluate the effects of incorporating powdered *caralluma* extract into wheat flour bread on various parameters. The study examined several criteria, including the measurement of total phenolics, antioxidant activity via DPPH and ABTS tests, inhibition of pancreatic lipase, alpha-amylase, and alpha-glucosidase, prevention of advanced glycation end product (AGE) formation, and sensory acceptability. The bread samples were categorized into four groups based on the amounts of *caralluma* extract powder used as raw materials: 100% wheat flour bread with CEP levels of 2%, 4%, and 6%.

### 3.1. Results of Sputum Specimen Inoculation into Liquid Media (Brain Heart Infusion (BHI) and Thioglycolate (THIO))

After incubation for 18–24 hours at 34–36°C, the results of inoculating 22 sputum specimens into BHI and THIO media showed turbidity, indicating bacterial growth. Initially clear, both media became cloudy, confirming positive bacterial proliferation, as illustrated in Figures 2 and 3. The presence of turbidity in the BHI and THIO media indicated successful bacterial isolation. This occurred because both BHI and THIO are enrichment media, designed to support bacterial growth by providing essential nutrients such as carbohydrates and proteins necessary for bacterial reproduction.

**Table 1** Results of Sputum Specimen Inoculation to BHI and THIO Media to Observe Bacterial Growth on BHI and THIO Media

| Patient | Media BHI | Media THIO |
|---|---|---|
| IS | + | + |
| AH | + | + |
| SU | + | + |
| PA | + | + |
| TU | + | + |
| SN | + | + |
| IN | + | + |
| AS | + | + |
| DA | + | + |
| YU | + | + |
| RU | + | + |
| PW | + | + |
| SK | + | + |
| SO | + | + |
| PO | + | + |
| SW | + | + |
| IW | + | + |
| ER | + | + |
| AA | + | + |
| AR | + | + |
| SR | + | + |
| PI | + | + |

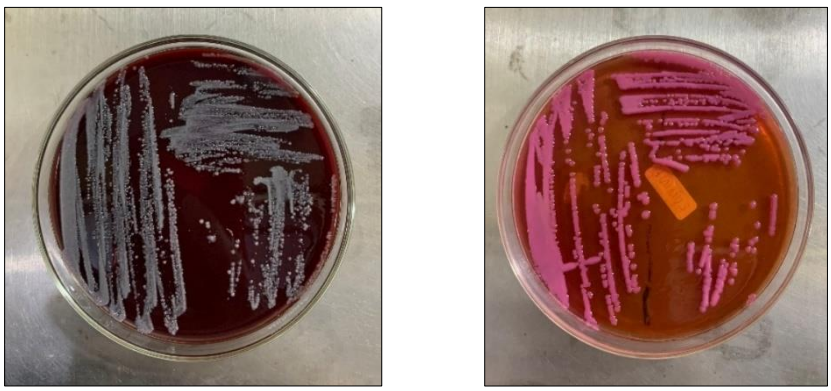
### 3.2. Results of Bacterial Growth from BHI Liquid Media to Solid Media of Blood Agar and MacConkey Agar (Streaking)

The results of bacterial streaking from BHI media onto blood agar and MacConkey agar, after incubation for 18–24 hours at 34–36°C, showed that all 22 specimens exhibited positive growth on blood agar, while only 11 specimens showed positive growth on MacConkey agar, and the remaining 11 were negative. The bacterial growth observed on blood agar is attributed to its differential nature, allowing the growth of a wide range of bacterial species [6]. In contrast, MacConkey agar is selective and supports the growth of only certain types of bacteria, particularly Gram-negative organisms [7]. Colonies on blood agar appeared in varying sizes large, medium, and small with a whitish-gray color, convex shape, shiny surface, and a red background. On MacConkey agar, colonies were round, pink, smooth, convex with even edges, and capable of fermenting lactose, indicated by their pink coloration.

**Table 2** Results of Bacterial Streaking from Isolate to Blood Agar and MacConkey Agar

| Patient | Media BHI | Media THIO |
|---|---|---|
| IS | + | - |
| AH | + | - |
| SU | + | + |
| PA | + | + |
| TU | + | + |
| SN | + | - |
| IN | + | + |
| AS | + | - |
| DA | + | + |
| YU | + | - |
| RU | + | + |
| PW | + | + |
| SK | + | + |
| SO | + | - |
| PO | + | + |
| SW | + | - |
| IW | + | - |
| ER | + | - |
| AA | + | + |
| AR | + | + |
| SR | + | - |
| PI | + | - |

Figure 1 presents the observational results of bacterial isolate streaking onto blood agar and MacConkey media, conducted to assess the morphological characteristics and growth patterns of bacterial colonies on each selective and differential medium."



Media Agar Darah (b) Media *MacConkey*

**Figure 1** The Results of Streaking/Plating Bacterial Isolates Showed the Growth of Bacterial Colonies

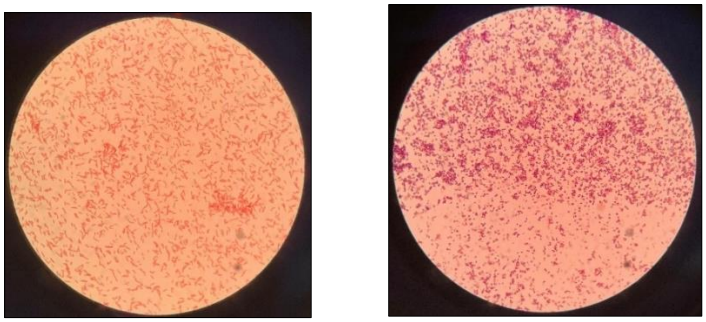
### 3.3. Results of Gram Staining

Gram staining was conducted on bacterial isolates obtained from blood agar and MacConkey media to determine the Gram characteristics of the organisms. This differential staining technique enabled the classification of isolates into Gram-positive and Gram-negative groups based on differences in cell wall composition. The results of Gram staining provided essential information for the subsequent identification and characterization of the bacterial species present in the sputum specimens.

**Table 3** Gram Staining Results from Blood Agar and *MacConkey* Media.

| Patient | Result | Shape | Color |
|---|---|---|---|
| IS | + | K | BK |
| AH | + | K | BK |
| SU | - | B | M |
| PA | - | B | M |
| TU | - | B | M |
| SN | + | K | BK |
| IN | - | B | M |
| AS | + | K | BK |
| DA | - | B | M |
| YU | + | K | BK |
| RU | - | B | M |
| PW | - | B | M |
| SK | - | B | M |
| SO | + | K | BK |
| PO | - | B | M |
| SW | + | K | BK |
| IW | - | B | M |
| ER | + | K | BK |
| AA | - | B | M |
| AR | - | B | M |
| SR | + | K | BK |
| PI | + | K | BK |

K: coccus, B: bacillus, BK: bluish purple, M: red



Gram Negative, Bacillus-shaped (b) Gram Negative, Cocci-shaped

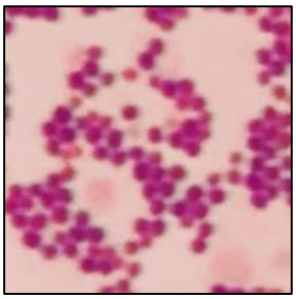
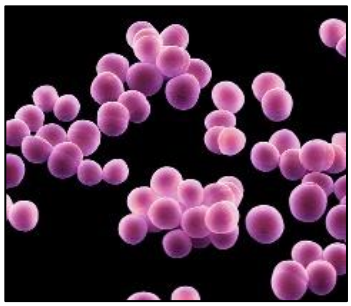
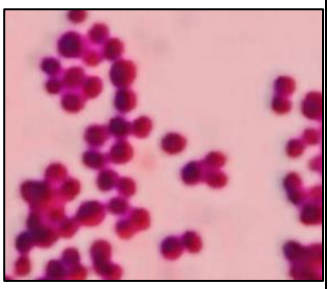
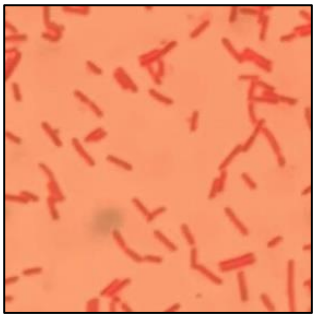
**Figure 2** Results of Gram Staining Observation with 100x Magnification

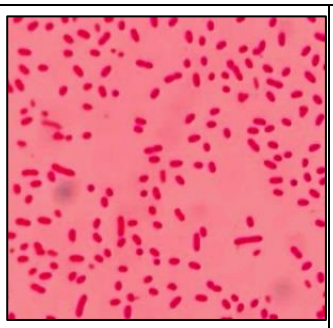
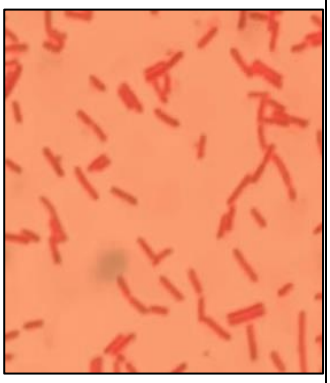
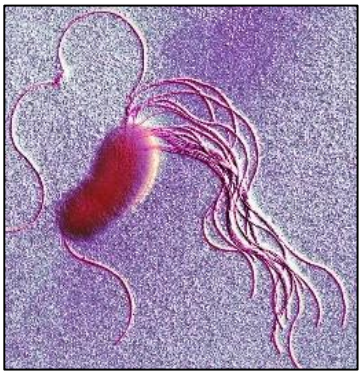
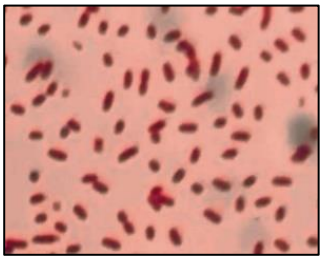
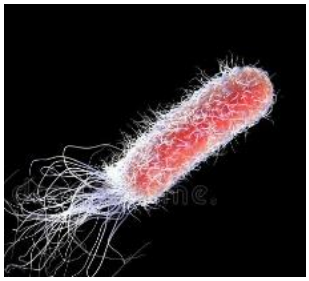
According to Table 3, Gram staining of 22 specimens resulted in 10 Gram-positive and 12 Gram-negative specimens. In Gram staining, cells that retain the crystal violet dye and appear bluish purple are classified as Gram-positive bacteria. In contrast, cells that lose the crystal violet and take up safranin, appearing red or pink, are classified as Gram-negative bacteria. The principle of Gram staining is based on the cell wall's ability to retain the primary stain (crystal violet) after decolorization with 95% alcohol. This characteristic is related to the composition of the cell wall. Gram-positive bacteria have a higher peptidoglycan content and lower lipid content compared to Gram-negative bacteria (8). Gram staining is performed to distinguish between Gram-positive and Gram-negative bacteria in order to determine the appropriate positive or negative panel for bacterial identification.

### 3.4. Bacterial Identification Results from Sputum Specimens of Pneumonia Patients

The identification of bacterial isolates from sputum specimens of pneumonia patients was carried out to determine the causative agents of the infection. The results of this identification, based on the analysis of bacterial growth on blood agar media and observed under a 100x magnification, are summarized in Table 4. This table presents a comprehensive overview of the bacterial species identified, offering insights into the microbial pathogens responsible for pneumonia in the studied patients.

**Table 4** Gram Staining Results from Blood Agar and *MacConkey* Media

| No | Results | Shape | Colour |
|---|---|---|---|
| 1. |  *Staphylococcus epidermidis* |  source: https://images.app.goo.gl/b4ReR6mz1xjMKbUNA | Round in shape, arranged in groups, purplish blue in color, and do not move because they do not have flagella. |
| 2. |  *Staphylococcus aureus* |  source: https://images.app.goo.gl/L1KptocNDEBHMwu97 | Round in shape, arranged in groups, purplish blue in color, and do not move because they do not have flagella. |
| 3. |  *Klebsiella pneumoniae* |  source: https://images.app.goo.gl/mjCUVtS3FGJqJ6ML9 | Rod-shaped, red in color, has a capsule, and does not move because it does not have a flagellum. |

| | | | |
|---|---|---|---|
| 4. |  <br>*Enterobacter aerogenes* |  <br>source: https://www.google.com/imgres?imgurl=https%3A%2F%2Fmedia.istockphoto.com | Rod-shaped, red in color and has flagella spread across the entire surface of the cell. |
| 5. |  <br>*Pseudomonas fluorescens* |  <br>source: https://images.app.goo.gl/LYKLSEyLSnWS32dq6 | Rod-shaped, red in color and has flagella that allow the bacteria to move. |
| 6. |  <br>*Pseudomonas aeruginosa* |  <br>source: https://www.google.com/imgres?imgurl=https%3A%2F%2Fthumbs | Rod-shaped, red in color, and have flagella that allow the bacteria to move. |

The genus *Staphylococcus* includes Gram-positive bacteria that are non-motile, facultative anaerobes, and require complex nutrients for growth. These bacteria are cocci-shaped, measuring 0.5-1.5 µm, and grow as small, shiny, pigmented colonies with a clear zone on blood agar [9]. The genus *Pseudomonas* is composed of Gram-negative, rod-shaped or cocci bacteria that are motile due to the presence of flagella, and they are obligate aerobes, meaning they require oxygen to grow [10]. The genus *Klebsiella* also belongs to the Gram-negative group and consists of short rod-shaped bacteria that are encapsulated, non-motile, and do not form spores. These bacteria typically measure 0.5-0.5 x 1.2 µm [11]. Finally, *Enterobacter* is a Gram-negative bacterium from the *Enterobacteriaceae* family. These bacteria are motile rods with smooth, white colonies and flagella evenly distributed on the cell surface, allowing for movement [12].

**Table 5** Results of Types of Bacteria Found Based on Age and Gender of Female Patients

| No. | Patient | Age | Type of Bacteria |
|---|---|---|---|
| 1 | YU | 44 years old | *Staphylococcus epidermidis* |
| 2 | SR | 47 years old | *Staphylococcus aureus* |
| 3 | PA | 52 years old | *Klebsiella pneumoniae* |

| | | | |
|---|---|---|---|
| 4 | AS | 53 years old | *Staphylococcus aureus* |
| 5 | IN | 61 years old | *Klebsiella pneumoniae* |
| 6 | IS | 63 years old | *Staphylococcus epidermidis* |
| 7 | IW | 63 years old | *Staphylococcus epidermidis* |
| 8 | SU | 70 years old | *Klebsiella pneumoniae* |
| 9 | ER | 78 years old | *Staphylococcus epidermidis* |
| 10 | RU | 94 years old | *Klebsiella pneumoniae* |

**Table 6** Results of Types of Bacteria Found Based on Age and Gender of Male Patients

| No. | Patient | Age | Type of Bacteria |
|---|---|---|---|
| 1 | SW | 48 years old | *Staphylococcus aureus* |
| 2 | PW | 49 years old | *Pseudomonas aeruginosa* |
| 3 | SN | 51 years old | *Staphylococcus aureus* |
| 4 | PI | 54 years old | *Staphylococcus aureus* |
| 5 | AA | 58 years old | *Klebsiella pneumoniae* |
| 6 | PO | 62 years old | *Klebsiella pneumoniae* |
| 7 | DA | 63 years old | *Pseudomonas fluorescens* |
| 8 | SO | 64 years old | *Staphylococcus aureus* |
| 9 | AR | 68 years old | *Pseudomonas aeruginosa* |
| 10 | TU | 72 years old | *Enterobacter aerogenes* |
| 11 | SK | 76 years old | *Klebsiella pneumoniae* |
| 12 | AH | 83 years old | *Staphylococcus aureus* |

Based on the findings from the research conducted at RSUD Jenderal Ahmad Yani Kota Metro, a total of 22 sputum specimens from pneumonia patients were analyzed to identify the types of bacteria presents. Out of the total, 10 specimens were collected from female patients (as shown in Table 5), revealing the presence of *Klebsiella pneumoniae* in 4 cases, *Staphylococcus epidermidis* in 3 cases, and *Staphylococcus aureus* in 2 cases. Meanwhile, 12 specimens came from male patients (as shown in Table 6), with *Staphylococcus aureus* identified in 5 specimens, *Klebsiella pneumoniae* in 3 specimens, *Pseudomonas aeruginosa* in 2 specimens, *Pseudomonas fluorescens* in 1 specimen, and *Enterobacter aerogenes* in 1 specimen. The results indicated a higher prevalence of pneumonia among male patients compared to female patients. This may be attributed to greater exposure to environmental risk factors, such as air pollution and tobacco smoke, which contain harmful chemical substances that can impair lung function and increase susceptibility to respiratory infections. In contrast, pneumonia in female patients may be influenced by poor dietary habits, overcrowded and unsanitary living conditions, and physiological states such as pregnancy, which can weaken the immune system and increase vulnerability to infections [13].

Furthermore, the study found that age played a significant role in the susceptibility to pneumonia. In female patients, those aged 44 years and above were more frequently affected, while in male patients, the risk increased from the age of 48 and above. This trend highlights the impact of age-related immune decline, which makes older individuals more susceptible to infections. Pneumonia risk is particularly high in children under the age of 2 and adults over 40 years of age. In infants, the respiratory system is still in the developmental stage, making it more vulnerable to infection. In contrast, in older adults, the immune system experiences a gradual decline in function, reducing the body's ability to fight off pathogens effectively. Elderly individuals, especially those aged 60 and above, are at a significantly higher risk due to this diminished immune response, making them more likely to develop serious respiratory illnesses, including pneumonia [13].

## 4. Conclusion

The results of this study indicate that the types of bacteria found in pneumonia patients varied according to age and gender. In terms of age, patients aged 44–62 years were found to have *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae*, while those aged 63–94 years exhibited a broader spectrum including *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Klebsiella pneumoniae, and Enterobacter aerogenes*. Based on gender, female patients were found to have *Klebsiella pneumoniae, Staphylococcus epidermidis, and Staphylococcus aureus*, whereas male patients showed greater bacterial diversity, with isolates such as *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, and Enterobacter aerogenes*. These findings suggest that both age and gender play a role in influencing the types of bacterial infections present in pneumonia patients.

## Compliance with ethical standards

*Disclosure of conflict of interest*

Authors have declared no conflict of interests.

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