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Detection of Bacteria from Sputum Samples in Patients Diagnosed with Pneumonia and Non-Pneumonia with Semiautomatic Tools

Chylen Setiyo Rini[1]*, Lenni Iffitawati[1]

[1]Jurusan Teknologi Laboratorium Medis, Fakultas Ilmu Kesehatan, Universitas Muhammadiyah, Sidoarjo 61215, Jawa Timur, Indonesia.

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***Corresponding author:**
chylensetiyorini@umsida.ac.id

Abstract

Pneumonia is an acute respiratory infection that affects the lung tissue (alveoli). Pneumonia can be caused by various microorganisms, including bacteria, protozoa, and viruses. Pneumonia affects around 450 million people each year. This study aims to identify bacteria from sputum cultures of patients diagnosed with pneumonia. This study uses a cross-sectional approach and is descriptive. The data used in this study were obtained from patient medical records through the electronic health record system of Bangil Regional Hospital. A total of 43 samples were analyzed, consisting of 35 sputum samples from patients who met the inclusion criteria for pneumonia diagnosis and 8 control samples. The samples were examined using a Mindray TDR 300B semi-automatic culture tool. The most frequently identified bacteria were *Raoultella ornithinolytica* and *Klebsiella oxytoca*.

INTRODUCTION

Pneumonia is a form of acute respiratory infection that attacks the lungs, the lungs consist of small sacs called alveoli that are filled with air. Patients with pneumonia, the alveoli contain pus and fluid that makes breathing difficult and oxygen is limited (WHO 2020). Symptoms of pneumonia caused by viruses can be more severe than symptoms of pneumonia caused by bacteria. Symptoms of this disease include shortness of breath and shortness of breath due to sudden lung infections (Kemenkes RI P2PTM 2018). Pneumonia is influenced by various factors, including age, cognitive status, comorbidities (including malignancies and chronic diseases). The most common bacteria from pneumonia are *Streptococcus pneumoniae* (Pnemococcus) and *Mycoplasma pneumoniae*, and viruses such as adenovirus, respiratory syncytial virus (RSV), influenza virus, rhinovirus and parainfluenza. Microbes causing pneumonia reach the lung tissue through the upper respiratory tract, infecting bronchioles and alveoli, triggering an inflammatory response that leads to alveolar and interstitial swelling (Anwar & Dharmayanti, 2014). Bacteria are one of the groups of prokaryotic organisms (do not have a nuclear envelope). Bacteria as living things certainly have genetic information in the form of DNA, but it is not found in a special place (nucleus) and there is no nuclear membrane. The form of bacterial DNA does not have introns and is only composed of axons. Bacteria also have extrachromosomal DNA which is combined into a small, circular plasmid. Bacteria are the germs that most often cause pneumonia (Brooks et al, 2004).

Sputum culture is a test that detects infectious bacteria or fungi in mucus. This procedure is recommended for patients suspected of having respiratory tract infections such as pneumonia, lung abscesses, or tuberculosis. Based on the Ministry of Health 2022, it explains that sputum or sputum examination can include culture and/or cytology examination of sputum or sputum samples. Sputum culture detects and identifies bacteria or fungi that cause infections such as pneumonia or pulmonary tuberculosis or the

respiratory tract leading to the lungs. Identification of antibiotics to treat infections (sensitivity testing) and monitoring treatment of infections (Ministry of Health 2022). Seeing the high mortality rate in pneumonia, pneumonia should be a problem that must be addressed and sputum culture is carried out to identify organisms to establish a definitive diagnosis. This study aimed to identify bacterial species isolated from sputum cultures of patients diagnosed with pneumonia.

METHOD

The design of this study is descriptive which explains the characteristics of bacterial names based on examination of sputum culture. This study was conducted at the microbiology installation of Bangil Pasuruan Regional Hospital. This study was conducted in March - May 2024, the ethical feasibility test was obtained from the ethics code division of Bangil Regional Hospital, Pasuruan with the number: 00.9.2/1404 / 424.072.01/2024. The population of this study was sputum from patients with a diagnosis of pneumonia at Bangil Regional Hospital, Pasuruan. There were 43 sputum samples from patients with inclusion criteria of sputum diagnosed with pneumonia without special criteria due to different patient conditions. The exclusion criteria were sputum from patients who were not diagnosed with pneumonia as controls. Samples were taken using accidental sampling technique where samples were taken by chance based on inclusion criteria during the study period, samples examined using Mindray tdr 300b culture mode semi-automatic microbiology tools, the examination began with checking the condition of the tool on the automated dosing system tool for automatic pipetting and microorganism analysis system, the presence of bubbles in the pipette. Bacterial colonies inserted into the common biochemistry medium bottle suspension to adjust the turbidity of the solution in the range of 0.45-0.5 *Mac farland* as a step to obtain a biochemical suspension. Then label the identity according to the patient's laboratory number. Perform inoculation on the test card according to the cartridge used and set the tool according to the test card used. Then drop mineral oil into the test card hole according to the needs of the cartridge used, and add mineral sugar oxidation if necessary according to the instructions in the test. Incubate the test card in an incubator at a temperature of ± 35^0C for 16-20 hours and observe the results according to the biochemical test standards (biochemistry test) and resistance test (susceptibility test). On the Mindray TDR 300 microorganism analysis system that will automatically read the test card and output the name of the bacteria on bacterial growth. The data obtained from the bacterial identification results were analyzed descriptively by observing the frequency and types of bacterial species identified in the sputum samples. No inferential statistical analysis was applied

RESULTS AND DISCUSSION

The test results can be collected automatically by the microorganism analysis system or the automatic identification and susceptibility test system, or observed manually into the analysis system, and the results of the strain identification and susceptibility will be calculated and stored in the analysis system. The identification test in the TDR method uses the appropriate type of reagent card (test card). The selection of the reagent card (test card) to be used is highly dependent on the morphology of the bacteria through colony identification on agar media and gram staining (Yingwu et al, 2006).

Table 1. Frequency distribution of bacteria cultured from sputum samples over a 3-month period with non-pneumonia and pneumonia diagnoses based on age.

Age	Diagnosa non pneumonia	Total	Diagnosa pneumonia	Total
0 - 9	-	0	Gram negatif	2
10 - 19	-	0	Gram negatif	0
20 - 29	Gram negatif	1	-	0
30 - 39	-	0	Gram negatif	2
40 - 49	Gram negatif	3	Gram negatif	6
50 - 59	Gram negatif	4	Gram negatif	11
			Gram positif	2
60 - 69	-	0	Gram negatif	4
70 - 79	-	0	Gram negatif	8
Total		8		35

Table 1 explains that from 43 samples during the study period, 8 samples were found as controls

with gram-negative bacteria types consisting of 2 samples aged 20-43 years and 6 samples aged 44-59 years. In the patient sample diagnosed with pneumonia, 35 samples were found with 33 gram-negative bacteria types and 2 gram-positive bacteria types. This is because diseases in the elderly are often different from young adults, because structural and functional changes in the respiratory system cause reduced body defenses. The body's defense mechanism is important against the entry of pathogens into the upper respiratory tract and respiratory tree, slower and less efficient clearance of secretions by the mucociliary system in the elderly has been shown to correlate with pneumonia (Jc, CHAN, and Hu 1994).

Table 2. Distribution of bacteria cultured from sputum samples over a 3-month period with non-pneumonia and pneumonia diagnoses based on gender

Gender	Non pneumonia (Person)	Presentase	Pneumonia (Person)	Presentase
Male	5	63%	22	63%
Female	3	37%	13	37%

Based on table 2, it is known that the number of non-pneumonia patients who are male is 3 people (37%) and female is 5 people (63%). While in pneumonia patients with male gender is 22 people (63%) and female is (37%). This is due to the physical differences in the anatomy of the respiratory tract between men and women, because in men the diameter of the respiratory tract is smaller, besides there are differences in endurance between men and women, and according to WHO (2001) men have higher excess activity compared to women in general, so they have more contact with dirty air (WHO 2001).

Table 3. Sputum culture sample results based on pneumonia and non-pneumonia diagnoses based on diagnosis

Non pneumonia Sampel control (8)	Name bacteria	Total sampel
CKD	*Acinobacter baumanii*	1
	Burkholderia cepacian	1
Fractue	***Raoultella ornithinolytica***	2
DM	*Klebsiella pneumonia*	1
Calculus kidney	*Burkholderia cepacia*	1
Dypsneu	*Klebsiella oxytoca*	1
ME	***Raoultella ornithinolytica***	1
Pneumonia Sampel (35)		
Pneumonia	***Raoultella ornithinolytica***	9
	Klebsiella oxytoca	7
	Klebsiella pneumoniae	3
	Eschericia coli	3
	Citrobacter gillenii	1
	Enterobacter aerogenesa	1
	Morganella morganii ss morganii	1
	Burkholderia cepacian	3
	Pseudomonas putida	1
	Strenophomonas maltophilia	1
	Pseudomonas fluorescens	2
	Enterobacter cloacae	1
	Staphylococcus aureus	1
	Staphylococcus intermedius	1

In Table 3, sputum culture examination of non-pneumonia and pneumonia diagnoses, both found the bacteria *R. ornithinolytica*. *Raoultella ornithinolytica* bacteria are Gram-negative encapsulated aerobic bacilli belonging to the Enterobacteriaceae family. Previously known as *K. ornithinolytica*, this bacterium was reclassified as *Raoultella* based on a new approach. *R. ornithinolytica* is mainly considered as an aquatic environmental bacterium and can be found in hospital environments (M et al. 2001). *R. ornithinolytica* infections are more frequently reported in the medical literature. This pathogen has the potential to cause various types of infections, including pneumonia. *R. ornithinolytica* bacteria that cause infections in humans including bacteremia, cholangitis, urinary tract infections, pneumonia, skin infections, osteomyelitis, meningitis, brain abscess, mediastinitis, pericarditis, conjunctivitis, otitis, and other infections (Klein and Flanagan 2016). *K. oxytoca* is one of several *Klebsiella sp*. bacteria. These bacteria are naturally found in the intestinal tract, mouth and nose. *K. oxytoca* is usually obtained from environmental sources and is a commensal pathogen in humans but is also an opportunistic pathogen that causes various infections (Yang et al. 2022). *K. pneumoniae* are opportunistic pathogenic bacteria that take advantage of decreased immune function to infect the human body. *K. pneumoniae* are bacteria that can live everywhere. These bacteria are transient flora found in the upper respiratory tract and skin. *K. pneumoniae* has been reported to be isolated from the environment such as surface water, and medical equipment. This bacteria is the cause of pneumonia, urinary tract infections, sepsis, meningitis and liver abscess (Virawan 2018). *Escherichia coli*, a gram-negative bacteria, is a member of the Enterobacteriaceae family and usually lives commensal in the digestive tract of humans and animals. However, it can acquire mobile genetic elements that provide virulence factors and allow it to become a pathogen that can cause pneumonia, diarrhea, enteritis, biliary tract infections, urinary tract infections, bacteremia, and neonatal meningitis (Alokasi, Masulli, and Alexeyev 2013). *E. coli pneumonia* is usually considered a nosocomial infection that occurs in patients with risk factors such as aspiration or mechanical ventilation. *B. cepacia* is an opportunistic pathogenic bacteria that often causes pneumonia in patients with immunocompromised conditions, including cystic fibrosis patients. Colonization or infection caused by *B. cepacia* is often referred to as cepacia syndrome, a severe and progressive pneumonia that can cause death due to septicemia or respiratory failure. Infections caused by *B. cepacia* generally occur as nosocomial infections in patients in contact with contaminated disinfectants and anesthetic equipment. Infections that occur include soft tissue, respiratory and urogenital tracts, but can also cause bacteremia, endocarditis and septic shock (Johnson and Russo 2002).

CONCLUSION

The analysis of 43 sputum samples from both pneumonia and non-pneumonia patients showed that 63% of pneumonia cases and 63% of non-pneumonia cases occurred in male patients, while 37% in both groups were female. This identical distribution raises the need for further clarification regarding the percentage calculations. Among all samples analyzed, the most frequently identified bacterial species were *R. ornithinolytica* and *K. oxytoca*. These findings support the aim of the study, which was to identify bacterial species isolated from the sputum cultures of patients diagnosed with pneumonia.

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