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Analysis of Deoxyribonucleic Acid Electrophoresis Results in Acute Respiratory Tract Infection Patients

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ABSTRACT

Introduction: Acute Respiratory Infection (ARI) is a term that refers to diseases that affect the upper respiratory tract (such as rhinitis, pharyngitis, and otitis media) and the lower respiratory tract (such as pneumonia, bronchitis, and strep throat) and can last up to 14 hours. The respiratory tract is made up of many organs, including the sinuses, pleura, middle ear, and nasal alveoli. **Objective:** Analyze the results of electrophoresis using agarose gel in patients with acute respiratory infections. **Method:** The research method used is experimental. **Results:** Based on the results of molecular tests using a documentary gel, it was found that 7 samples had positive DNA of the ply *S. pneumoniae* gene, while 348 bp DNA was found to have negative results. **Conclusion:** The frequency distribution of respondents from 10 samples used electrophoresis equipment and gel documentaries as a means of detecting 7 positive samples. A positive result is stated if the gel documentary shows the DNA of the tape. The size of the DNA band determined by the DNA marker located at 348 bp corresponds to the target or sample. This shows that the DNA of the sample has a size of about 348 bp.

Keywords : ARI, electrophoresis, streptococcus pnuemoniae

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INTRODUCTION

Acute Respiratory Tract Disease (ARI) is a disease of the upper respiratory tract (such as rhinitis, pharyngitis, and otitis media) and the lower respiratory tract (such as bronchitis, pneumonia, and sore throat) that can last for 14 days. This term is adapted from the English term Acute Respiratory Infections (ARI). Many organs form the respiratory tract, including the nasal alveoli, sinuses, middle ear space, and pleura [1]. More than 300 types of bacteria, viruses, and rickettsiae can cause ARI. The bacteria that cause ARI are Streptococcus, Staphylococcus, Hemophilus, Bordetella, Corinebacterium, and Pneumococcus. The viruses that cause ARI are Picornavirus, Herpesvirus, Myxovirus, Adenovirus, and Mycoplasma [2].

According to the World Health Organization [3], ARI is a major cause of death (morbidity) and illness worldwide. Around four million people die each year from ARI, with 98% of these deaths related to lower ARI. In low- and middle-income countries, the mortality rate for infants, young children, and adults is very high.

As shown by the Basic Health Survey conducted in 2018, the province of East Nusa Tenggara has the highest incidence of ARI at 15.4 cases per 1000 population. Behind it is Papua with 13.1 cases per 1000 population, West Papua with 12.3 cases per 1000 population, Banten with 110,000 population and Bengkulu with 11.8 cases per 1000 population. North Sumatra Province is ranked 30th with an ARI incidence rate of 6.8 per 1,000 population [4].

According to the Medan City Health Office, the incidence of ARI increases every year. In 2012, the highest

incidence was 47.5%; in 2013, the figure continued to increase to 38.4%; in 2014, the figure continued to increase to 46.1%; and in 2015, the figure continued to increase to 39.87%, 40.23%, and 39.98% in 2017 [5]. The number of ARI cases in Medan City increased to the third highest in 2020. (BPS SUMUT, 2020) Previous research [6] found that ARI infection causes swelling of the mucosa of the respiratory tract walls and narrowing of the respiratory tract. The agent irritates, damages, stiffens or slows down the movement of hair (cilia) so that the cilia cannot sweep mucus and foreign objects in the respiratory tract. Hypersecretion is a reaction that occurs when agents settle in the mucosa-producing tract. If this happens to children, excessive mucus will come out of the nose because the mucus transport capacity has exceeded its limit. One of the symptoms of ARI is coughing and mucus coming out of the nose.

A study conducted [7] found that out of 100 patient samples, 21 *S. pneumoniae* isolates were found using the PCR method. Male patients were 61% (61 out of 100), and female patients were 39% (39 out of 100). Most patients were over 51 years old. Then the samples collected were traced through medical records. 20% of patients with a primary diagnosis of pneumonia, 29% with a secondary diagnosis of pneumonia, and 51% of patients with a diagnosis of other respiratory tract infections conducted research with sputum samples.

Based on research by [8] many factors cause errors in sputum sampling, one of which is the incorrect way of expelling sputum (only a small cough) so that the expected sputum does not really come out of the deep bronchus. Given the many factors that cause errors in sputum sampling, the author is interested in conducting research using oropharyngeal (throat) swab samples using electrophoresis examination on agarose gel.

MATERIALS AND METHODS

Tools and Materials

The tools used in this study were Cryotube, Tip, Hot Plate, Plastic clip, Autoclave, Beaker Glass, Erlen Meyer, VTM swab, Micro pipette, Analytical balance, Stirring rod, Petri dish, Electrophoresis, Incubator. The materials used in this study were agarose, TAE/TBE buffer, distilled water, loading buffer, BAP media, H2O2 solution, PBS solution, EtBr.

Taking Oropharyngeal Swab

The steps for taking oropharyngeal swab are to use standard PPE. Prepare a cryotube containing 1.5 ml of virus transport media. Then use a swab made of sterile Dacron/rayon with a plastic stem. Wipe the back of the tonsils and avoid the swab touching the tongue. Then insert the oropharyngeal swab as soon as possible into the cryotube containing the virus transport medium. Break the plastic stem in the mouth area of the cryotube so that the cryotube can be closed tightly. The cryotube is then wrapped in parafilm. Then the cryotube containing the swab is wrapped in clean tissue and then inserted into a plastic clip. After that, store it at a temperature of 4-8 ° C before examination.

Making BAP Media

Dissolve 40 gr of agar base in 1000 ml of distilled water in a bottle. Then stir until smooth, put it in an autoclave at a temperature of 121 ° C for 15 minutes. After being autoclaved, let it cool to 47 ° C, then add 5% goat blood, then homogenize and pour into a petri dish. Let it solidify and BAP is ready to use.

Isolation on BAP

The bacterial suspension is isolated on BAP media and then incubated in a CO^2 incubator at a temperature of 35-37 ° C with a CO^2 gas concentration of 5% for 18-24 hours.

Catalase Test

A 30% H_2O_2 solution is dropped on the surface of the colony. *S. pneumoniae* is characterized by the absence of gas bubbles after being dripped with H_2O_2.

Colony Collection and DNA Isolation of *S. pneumoniae*

The *S. pneumoniae* colonies that grew on BAP were harvested. The colonies were put into 200 µl of PBS solution and then heated at 90°C for 30 minutes.

Making TAE/TBE Buffer

The *method* for making TAE/*TBE* buffer is as follows, make a working solution of 1x TAE buffer, take 100 ml of 10x TAE buffer, dissolve in 900 ml of distilled water. Then stir until smooth to get 1000 ml of 1x TAE buffer solution.

Preparation of Agarose Gel

Weigh 0.4 gr of agarose. Then dissolve it into 1x 40 ml of TAE buffer. Heat on a hotplate, then cool. Then add 4 ul of EtBr, cool for a while, pour into the gel tray mold. Install the comb, let it harden, remove the comb. Change the gel position from casting to running. Pour 1x TBE buffer into the chamber until the gel is submerged approximately 2-3 mm.

Electrophoresis Working Procedure

After the gel and wells are formed, insert the agarose gel into the electrophoresis, fill the left and right containers with 1x TAE buffer solution as an electrolyte solution. Then insert 5 µl of DNA sample mixed with 1x dye/loading buffer solution into the well. Connect to the power supply and turn on at 100 volts for 2 hours. Then press the run button to start. After completion, the agarose gel is soaked in ethidium bromide solution for 15 minutes and soaked 2 times with distilled water until clean. Genomic DNA is analyzed above the transilluminator using ultraviolet light with a gel documentary tool, where positive samples are marked by the formation of a 348 bp ply gene band.

RESULTS AND DISCUSSION

Results

Table 1. Frequency Distribution of Respondents Based on Age, Gender, Occupation and Education

No	Age	F	%
1	18-40	4	40
2	>41	6	60
	Total	10	100
No	**Gender**	**F**	**%**
1	Male	8	80
2	Female	2	20
	Total	10	100
No	**Occupation**	**F**	**%**
1	Civil Servants	2	20
2	Employee	5	50
3	Farmer	2	20
4	Housewife	1	10
	Total	10	100
No	**Education**	**F**	**%**
1	Junior High	3	30
2	Senior High	4	40
3	S1	3	30
	Total	10	100

Among the 10 respondents, the frequency distribution table of characteristics of pneumonia patients in Lubuk Pakam District shows that the majority of pneumonia patients are over 41 years old, as many as 6 people (60%), the majority of gender is male, as many as 8 people (80%), the majority of respondents about their jobs are employees, as many as 5 people (50%), and the majority of respondents about their education are high school, as many as 4 people (40%).

Table 2. Frequency Distribution of Respondents of Positive Pneumonia Patients in Lubuk Pakam District

No	Electrophoresis Results On The Documentary Gel Of The Ply Gene Of *S. pneumoniae*	F	%
1	Positive	7	70
2	Negative	3	30
	Total	10	100

The frequency distribution table of respondents made based on the molecular electrophoresis method with documentary gel shows that 7 out of 10 respondents were positive for *S. pnuemoniae*. Of the ten samples planted on BAP media*, S. Pnuemoniae* has the characteristics of clear, mucoid, waterish, and grayish colonies. In addition, it shows alpha hemolysis activity. The catalase isolate test shows that seven isolates do not produce gas bubbles after being dripped with H_2O_2. This indicates that the isolate contains *S. pnuemoniae*.

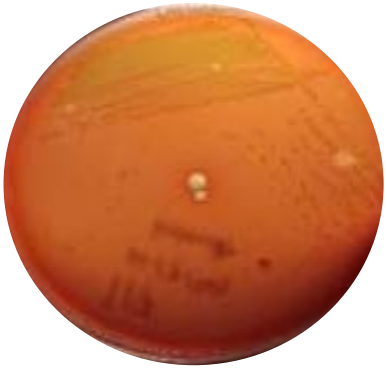

Figure 1. Image of a colony of *Streptococcus pneumoniae* on BAP

Based on the molecular results using documentary gel (Figure 2), the DNA band of the ply gene of *S. pneumoniae* was detected in 7 samples which were declared positive because there was a 348 bp DNA band and negative results were obtained in 3 samples.

Figure 2. Image of amplification results from the ply gene product of Streptococcus pneumoniae with a 348 bp marker. Description: M = Marker, K + = Positive control, K- = Negative control and S = Sample

Table 3. Results of Identification of *S. pneumoniae* Bacteria from Oropharyngeal Swab Samples of Pneumoniae Patients Using the Electrophoresis Method

Metode	Marker	Positive	Negative
Elektrophoreses	348 Bp	7	3

Based on the table above, all samples tested molecularly, 7 out of 10 samples were positive for *Streptococcus pneumoniae* bacteria.

DISCUSSION

In *this* study, a molecular examination was carried out using the electrophoresis method using agarose gel. The principle of electrophoresis is the movement of charged molecules or ions in a semi-solid medium under the influence of an electric field and can be used to determine the size of DNA using a known size marker [4].

The *electrophoresis* technique is influenced by the selection of the separating medium. There are two media that are often used in using electrophoresis. The first is Agarose gel, which is a standard method for identifying and purifying Deoxyribo Nucleic Acid (DNA) and Ribose Nucleic Acid (RNA) fragments. The advantages of this gel are easier, simpler and the rate of separation is faster to form fragments and is non-toxic [4].

The research sample was an oropharyngeal swab sample from a patient with pneumonia taken from the GrandMed Lubuk Pakam hospital. A total of 10 oropharyngeal swab samples of pneumonia patients with 8 male and 2 female.

Frequency distribution of characteristics of respondents of positive pneumonia patients in the district. Lubuk Pakam of 10 respondents, the majority of pneumonia patients were >41 years old, 6 people (60%), the majority of respondents were male, 8 people (80%), and the majority of respondents' jobs were employees, 5 people (50%).

This is in accordance with several other researchers who found that pneumonia is most often found in the elderly and pneumonia sufferers are more common in men than women. People who are elderly have a high risk of getting pneumonia, this is because in the elderly age group, the body's immunity tends to weaken so that the possibility of being exposed to Streptococcus pneumoniae bacteria is greater [9,10,11].

The level of education also affects pneumonia prevention behavior. The lower a person's level of education, the more difficult it will be to understand pneumonia and this will affect pneumonia prevention behavior. Education is an effort to provide knowledge to increase positive behavioral changes, knowledge influenced by the level of education is one of the triggering factors that plays a role in influencing a person's decision to behave healthily, the higher a person's level of education, the better their knowledge about pneumoniae so that control so as not to be infected and treatment efforts if infected are also maximized [12 - 16].

The most potential environment for transmission outside the home is the environment or workplace because of its specific environment with a population concentrated at the same time, workers generally live around dense settlements and unhealthy environments [17].

Of the 10 respondents, 7 respondents (70%) were found to be positive for pneumoniae using the electrophoresis method, the results of this study are in line with research stating that Streptococcus pneumoniae examination has good sensitivity, where in molecular examination using the electrophoresis method can read the bp level in Streptococcus pneumoniae bacteria using the Streptococcus ply gene marker pneumoniae [18,19].

These results are in accordance with expectations where in the diagnostic test tool which is mainly to determine the presence or absence of a disease, high sensitivity and specificity values are expected so that it will ensure the diagnosis of pneumonia sufferers [20].

CONCLUSIONS

Based on the results of research on the analysis of electrophoresis results on oropharyngeal swabs in patients with acute respiratory infections, it can be concluded that frequency distribution of respondents from 10 samples using electrophoresis and documentary gel as readers detected 7 positive samples. Positive results are stated if the results in the documentary gel find DNA bands. The size of the DNA band produced with the DNA marker which is at a size of 348 bp, according to the target/sample. This shows that the DNA band in the sample has a size of around 348 bp.

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