Chapter 1

Introduction

1.1 Background

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, opportunistic pathogen that is abundant in natural and clinical environments, including soil, water, and hospital settings. Despite its common distribution in the environment, *P. aeruginosa* stands out due to its problematic resistance mechanisms, and ability to cause chronic respiratory tract, urinary tract, bloodstream, ear, eye, and wound infections, including those that occur in diabetic patients (Al-Dahmoshi et al., 2020; Elfadadny et al., 2024). In these cases, pathogenic *P. aeruginosa* delays tissue healing, increases the risk of complications, and amplifies hospitalization and amputation risks (Rodríguez et al., 2021). Furthermore, these infections are often complicated by biofilm formation and delayed identification of the pathogen, which may limit antibiotic penetration (Yin et al., 2022). Considering the potential negative effects of *P. aeruginosa* and time consuming conventional diagnostics, it is important to develop alternative treatment methods and rapid diagnostic kits.

In Indonesia alone, there has been an increase in the number of diabetic patients, with the number of individuals suffering from diabetes estimated to be 18.69 million in 2020 and is expected to continue increasing to 40.7 million in the year 2045 (Wahidin et al., 2024). Recent reports show that diabetes is increasingly being diagnosed in younger adults and even adolescents, raising concerns about long-term complications (Haryanti, 2026). This increasing trend is concerning because diabetic patients are highly susceptible to chronic wound infections, including diabetic foot ulcers, which are frequently associated with opportunistic pathogens such as *P. aeruginosa* (Garousi et al., 2023). The increasing number of diabetic patients may lead to a greater demand for effective antimicrobial strategies capable of treating chronic wound infections caused by antibiotic-resistant bacteria.

An emerging potential for antibacterial treatment and detection is bacteriophages or commonly known as phages, which are viruses that specifically infect bacteria (Mathieu et al., 2019). Lytic phages can quickly and selectively kill target bacteria, including multidrug resistant strains without heavily disrupting the surrounding microbes unlike conventional antibiotics. Furthermore, binding proteins engineered from phages allow for highly specific, sensitive and rapid diagnostics (Peng et al., 2024; Guo et al., 2021). Additionally, because phages naturally evolve with their bacterial hosts, they can adapt to the changing surface receptors and resistance mechanisms, further increasing their potential effectiveness against antibiotic resistant bacteria (Hasan & Ahn, 2022). Considering their abundance, environments such as river systems represent a rich source of phages due to the frequent input of human, agricultural, and hospital-associated bacteria, making them suitable starting points for isolating lytic phages that can infect pathogenic bacteria such as *P. aeruginosa*.

Despite the growing interest in phage research, the availability of well-characterized phages targeting *P. aeruginosa* remains limited in Indonesia (Kapoor et al., 2024). Because phages naturally co-evolve with their regional bacterial hosts, exploring local environment reservoirs, such as river systems, is a crucial step for discovering indigenous phages. Isolating and characterizing these local phages will not only address the research gap but also provide explorative data to support the future development of targeted therapy for DFU management in Indonesia.

1.2 Objective

The general objective of this study is to isolate and characterize bacteriophages obtained from river water samples that are capable of infecting *Pseudomonas aeruginosa* and evaluate their potential antibacterial activity. The specific objectives include:

- To isolate bacteriophages from river water samples capable of infecting *Pseudomonas aeruginosa.*
- To amplify and quantify the isolated bacteriophages using plaque assays and determine their phage titers (PFU/mL).
- To observe the plaque morphology of the isolated bacteriophages on agar plate.
- To evaluate the lytic activity of the isolated bacteriophages against *Pseudomonas aeruginosa* using *in vitro* killing assays at different multiplicities of infection (MOI).
- To determine the stability of the isolated bacteriophages when exposed to commonly used antiseptics and disinfectants.
- To assess the ability of the isolated bacteriophages to inhibit and eradicate *Pseudomonas aeruginosa* biofilms.
- To evaluate the lytic spectrum of the isolated phage against sensitive and carbapenem-resistant *P. aeruginosa* (CRPA) clinical isolates

1.3 Hypothesis

H_0 (Null Hypothesis):

Bacteriophages isolated from river water samples do not show significant lytic activity against *Pseudomonas aeruginosa*, do not significantly reduce bacterial growth *in vitro*, and do not significantly inhibit or eradicate *Pseudomonas aeruginosa* biofilms,and do not show broad host range infectivity against multiple *Pseudomonas aeruginosa* strains, including carbapenem-resistant isolates.

H_1 (Alternative Hypothesis):

Bacteriophages isolated from river water samples show significant lytic activity against *Pseudomonas aeruginosa*, reduce bacterial growth *in vitro*, indicate measurable inhibitory or eradication effects on *Pseudomonas aeruginosa* biofilms, and exhibit broad host range infectivity against multiple *Pseudomonas aeruginosa* strains, including carbapenem-resistant isolates.