Chapter 1

Introduction

1.1 Background

In 2019, Indonesia had approximately 10.7 million people living with diabetes and is projected to increase to 16.6 million by 2045, representing one of the highest absolute prevalence rates worldwide. Diabetes mellitus was also among the top three causes of death in Indonesia in 2017 (Hidayat et al., 2021). One of the most severe complications of diabetes is impaired wound healing driven by chronic hyperglycemia. As a result, diabetic foot ulcers are common complications among patients with diabetes, with approximately 50–60% of these ulcers becoming infected, and around 20% of moderate to severe infections ultimately leading to lower-extremity amputations (Zhang et al., 2016).

Diabetic foot infections (DFIs) infections are commonly polymicrobial, with *Staphylococcus aureus* (*S. aureus*) being the most frequently isolated pathogen (Radji et al,. 2014; Sultana et al., 2023). The pathogenicity of *S. aureus* is largely attributed to its variety of virulence factors and toxins that facilitate tissue invasion, immune evasion, and persistent infection (Dunyach-Remy et al., 2016). Furthermore, *S. aureus* is capable of forming biofilms which allows it to persist on wound tissues, contributing to delayed healing, recurrent infections, and increased difficulty in treatment (Tuon et al., 2023).

In clinical practice, bacterial identification in diabetic wound infections is commonly performed using conventional culture-based methods, including wound swab cultures and blood cultures. Although these techniques are considered reliable for pathogen identification, they are often time-consuming and may require several days to generate results (Matheson et al., 2021; Peters, 2016). During this period, patients are frequently treated with broad-spectrum antibiotics before the causative pathogen is definitively identified. This practice can contribute to the inappropriate use of antibiotics and accelerate the development of antibiotic-resistant bacteria (Tran et al., 2024; Senneville et al., 2023). Additionally, *S. aureus* has increasingly shown multidrug-resistant (MDR) profiles, including the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA). The resistant strains complicate treatment and are associated with prolonged infections (Boulton et al., 2020). Due to the growing prevalence of antibiotic resistance, alternative antibacterial strategies for managing infections caused by resistant pathogens are needed.

Bacteriophages have emerged as a promising alternative. Bacteriophages, also known as phages, are viruses that specifically infect and replicate within bacterial hosts (Thi et al., 2025). Their high host specificity and rapid phage host interactions enable bacterial recognition and lysis within a short time frame making it attractive for both therapeutic and diagnostic applications. In particular, lytic bacteriophages can be exploited as an alternative therapy for treating bacterial infection and detecting specific bacterial pathogens (Abedon, 2025).

Environmental waters, including rivers and wastewater systems impacted by urban, industrial, and clinical discharges, serve as significant reservoirs of antibiotic-resistant bacteria. Urban aquatic environments have been reported to harbor multidrug resistant *Staphylococcus* species and other clinically relevant resistant bacteria, reflecting anthropogenic pollution and the dissemination of antibiotic residues into natural water systems (Velazquez-Meza et al., 2024). Importantly, these environments are also rich sources of bacteriophages. Sewage and wastewater, in particular, have been widely recognized as ideal reservoirs for isolating phages that infect clinically important bacteria, as they concentrate both bacterial hosts and their corresponding phages (Aghaee et al., 2021; Ahmad et al., 2025). Previous studies have demonstrated the potential of *S. aureus*-targeting bacteriophages for the treatment of diabetic foot infections and other chronic wound infections. A study done by Plumet et al. (2025) reported that bacteriophages were able to effectively degrade established *S. aureus* biofilms and promote wound healing in chronic diabetic foot infection models. These findings highlight the potential of bacteriophages as an alternative strategy for managing biofilm-associated infections. Furthermore, the isolation of local bacteriophages is important because phage-host interactions can vary across geographical regions and bacterial populations, making locally sourced phages potentially better adapted to infect circulating bacterial strains (Hyman, 2019). This is particularly relevant in Indonesia, where studies investigating therapeutic bacteriophages against clinically important pathogens remain limited. However, limited studies have explored the isolation and functional characterization of Staphylococcus-targeting bacteriophages from wastewater in Indonesia for treating DFIs.

1.2 Objective

This study aims to isolate and functionally characterize *Staphylococcus aureus*-targeting bacteriophages from wastewater sources in Jakarta, Indonesia. Furthermore, this study aims to evaluate the biological properties of the isolated bacteriophages, including their infectivity, stability, antibacterial activity, and morphological characteristics, in order to better understand their potential for applications against *Staphylococcus aureus*.

1.3 Hypothesis

H_0: Bacteriophages isolated from wastewater do not demonstrate significant infectivity against *Staphylococcus aureus*, do not effectively reduce bacterial growth in vitro, do not inhibit or eradicate *S. aureus* biofilm formation, and do not exhibit stable or consistent antibacterial activity under tested conditions.

H_1: Bacteriophages isolated from wastewater demonstrate significant infectivity against *Staphylococcus aureus*, effectively reduce bacterial growth in vitro, inhibit and eradicate *S. aureus* biofilm formation, and exhibit measurable antibacterial activity and stability under tested conditions.