UNIVERSITI TEKNOLOGI MARA

GENETIC DETERMINANTS OF *Staphylococcus haemolyticus* IN COMMENSAL AND CLINICAL ISOLATES

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ABSTRACT

In this study, the genetic determinants in both commensal and clinical isolates of *S. haemolyticus* which includes Multi-Drug Resistance (MDR), Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing and biofilm formation were characterized. A total of 50 commensal and 98 clinical isolates of *S. haemolyticus* were successfully isolated and tested against eleven types of antibiotics. From these, 40.0% of the commensals were MDR strains while a higher percentage of 69.4% of the clinical isolates were also MDR strains. The antibiotic profile data indicates the natural resistance ability of *S. haemolyticus* against killing by antibiotics regardless whether they are from commensal or clinical isolates. It also shows that MDR strains are more prominent among the clinical strains than the commensals. In contrast, all the isolates regardless of commensals or clinical were susceptible against vancomycin suggesting that this antibiotic is suitable for treatment in *S. haemolyticus* infections. SCC*mec* Type II was found to be a dominant typing for both commensal and clinical isolates at 90.0% and 98.99% respectively followed by Type V, Type I, Type IV and Type III. Similar pattern of typing observed indicates the possibility that the clinical isolates of *S. haemolyticus* could probably originated from the commensals strains that had successfully enter the host and caused infections. Unlike *S. epidermidis*, the majority of *S. haemolyticus* did not form biofilm on micro-titre plate nor did they harbour the *ica*AD gene. This suggests that biofilm formation may not be a virulent factor for the pathogenicity of *S. haemolyticus*. Isolate E1, S90 and A114 were further subjected to *de novo* Next Generation Sequencing (NGS). The results revealed that these isolates harbour eleven antibiotics resistance genes, nine SCC*mec* sequences, sequences homologous to two phages and nine plasmids, and they also carry the IS1272 insertion sequences. Hence, it appears that these genetic determinants are widespread among the *S. haemolyticus* isolates and may serve as "vehicles" for the transmission of antibiotics resistance. However, there is not enough evidence to conclude if an isolate can progressively become resistance to more antibiotics by sequentially accumulating these genetic determinants tested.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Some bacteria which exist as normal flora are thought to be harmless. These bacteria are known as commensals where they benefit from the host while the host is neither benefit nor harmed (Bogitsh *et al.*, 2019). However, it was later revealed that some of these commensals thought to be harmless have a huge potential as infectious pathogens instead and become resistant against the most known antimicrobial drugs (Becker *et al.*, 2014). One example of normal flora bacteria with such capabilities is *Staphylococcus* sp. (Adegoke and Okoh, 2012; Brown and Horswill, 2020).

In the latest 2020 updates, *Staphylococcus* comprises around 60 species which most of them can be found abundantly as commensals on human and animals (Coates-Brown *et al.*, 2018; Asante *et al.*, 2020). Depending on their respond against coagulase, *Staphylococcus* can be classified into two major groups of Coagulase Positive *Staphylococcus* (CoPS) and Coagulase Negative *Staphylococcus* (CoNS). Coagulase is an extracellular enzyme which binds to prothrombin in the host's cells to form staphylothrombin that converts fibrinogen into fibrin by forming a localized clot (Foster, 1996). Hence, coagulase is a traditional marker to differentiate between both CoPS and CoNS in clinical microbiology studies (Argemi *et al.*, 2019).

The most pathogenic species reported among the staphylococci is *Staphylococcus aureus*, a member of CoPS which is known to be one of the main causative agents for nosocomial infections (Becker *et al.*, 2020). Depending on the site of infection, this species can cause invasive infections or toxin-mediated diseases (Taylor and Unakal, 2021). In 2015, it was documented that around 500,000 patients in the United States of America (USA) hospitals were infected by staphylococcal infections, mainly because of Methicillin-Resistant *S. aureus* (MRSA) with up to the 10.0% of mortality rate (Tong *et al.*, 2015).

CoNS, on the other hand, was historically assumed to be non-pathogenic (Rossi *et al.*, 2020; Becker *et al.*, 2020). Conversely, they now epitomize as one of the major causative agents for hospital acquired infections (Lu *et al.*, 2020). Among the most predominant species of CoNS include *S. epidermidis*, *S. haemolyticus*, *S. capitis* and *S.*