UNIVERSITI TEKNOLOGI MARA

INTEGRATIVE AND CONJUGATIVE ELEMENTS MEDIATE TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN *HAEMOPHILUS INFLUENZAE* THROUGH CONJUGATION MECHANISMS

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ABSTRACT

Haemophilus influenzae is a distinct member of the *Haemophilus* spp. They are classified into serotypable (type a-f) and non-typeable strains. The widespread uses of a conjugate vaccine against the highly pathogenic *H. influenzae* type b (Hib) had successfully limited the diseases. However, other serotypes such as *H. influenzae* type a (Hia) and non-typeable *H. influenzae* (NTHi) had emerged and became more prominent pathogen over the last few decades, causing diseases almost similar to Hib. One of the virulence factors necessary for bacterial survival is antibiotic resistance, and it became too familiar nowadays, including amongst *H. influenzae*. Most commonly, bacterial resistance genes are harboured by plasmids, either in stable form or larger, chromosomally integrated plasmid known as integrative and conjugative elements (ICE). This study aimed to identify the ICE in antibiotic resistance NTHi and observe the transfer of ICE to Hia through the conjugation mechanism. Resistance to antimicrobial drugs was determined using a standard disk diffusion method involving ampicillin, tetracycline, and co-trimoxazole, and later confirmed by PCR detection of the related genes. ICE was also identified by using PCR-gel electrophoresis detection of the specific determinants in its sequence. The conjugative transfer of ICE between different strains was carried out using the cell mating technique and confirmed by transconjugant colony formation on selective agar. Whole-genome re-sequencing was done to analyze the genome of the transconjugant cells upon the acquisition of ICE. The analyses were carried out using several bioinformatics tools to compare the obtained genetic sequences with the available database. Overall, 8/14 (57%) of NTHi and 2/4 (50%) of Hia were resistant to at least one of the antibiotics tested, and related resistance genes were detected in >90% of the resistant strains. ICE was detected in 5/8 (63%) of those antibiotic resistance NTHi and none for Hia. Based on these findings, NTHi H620 strain and Hia H86 were selected as donor and recipient for cell mating, respectively. However, ICE's conjugative transfer between these strains was experimentally unsuccessful, as indicated by the absence of transconjugants on the selective agar. Nevertheless, the experiment was remodelled by using a similar strain NTHi H152 as the recipient. As a result, transconjugant colonies were formed after overnight incubation, and thus, the colonies were harvested and proceeded with DNA extraction. PCR amplification detected ICE and the donor's resistance gene (*bla*TEM-1) as well as the recipient's resistance genes (*dfr*A1) in the transconjugant DNA. Upon bioinformatics analyses, it was evident that the transconjugant's ICE originates from the donor strain, carrying the *bla*TEM-1 gene in its transposon. The mobilization of antibiotic resistance genes through cell-to-cell conjugation in prokaryotes is not uncommon and has been reported in numerous studies worldwide. Only these recent years, scientists were aware of the role of ICE in this process apart from stable, standalone plasmids', which could explain the increasing cases of antibiotic-resistant bacteria these days. The unsuccessful transfer of ICE between NTHi and Hia in this study may be caused by a few underlying factors not fully understood yet. Nevertheless, future studies may investigate loopholes in this research to elucidate the actual mechanisms in resistance genes transfer and provide valuable insights in combating this problem.

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CHAPTER ONE
INTRODUCTION

1.1 Research background

Haemophilus influenzae is among the leading causes of upper and lower respiratory tract infections in children and adults (Erwin et al., 2005; Rubach et al., 2011). This bacterium is morphologically small (1 μm × 0.3 μm), pleomorphic and gram-negative coccobacilli with random arrangements. They grow best at 35-37°C with 5% CO_2 and require hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth. Some strains of *H. influenzae* possess a polysaccharide capsule surrounding the cell, which can be further grouped into six different serotypes (a-f) based on the type of capsular antigen expressed (De Chiara et al., 2014). Of all serotypes, *H. influenzae* serotype b (designated as Hib), which expresses polyribosyl ribitol phosphate (PRP) capsular antigen, is considered the most virulent serotype due to its ability to invade the mucosal epithelium and resist the actions of the host immune system. Some *H. influenzae* serotype strain a (designated as Hia) were also found to cause invasive infections, particularly throughout the decades of post-Hib conjugate vaccine implementation (Tsang & Ulanova, 2017).

However, most *H. influenzae* in the normal microbiota of the upper respiratory tract are not encapsulated (i.e., no identifiable capsular polysaccharides), and the strains are referred to as non-typeable *Haemophilus influenzae* (designated as NTHi) (Balows et al., 2013). Although NTHi is not as highly rated as the invasive Hib strains, they are the common cause of upper respiratory tract infections in children and adults (Wang et al., 2011). The infections caused by NTHi are primarily non-invasive and mucosal in nature, such as otitis media, sinusitis, conjunctivitis, bronchitis, and pneumonia. However, over the last decade, the cases of invasive NTHi infections are increasing in number as reported in several studies based in England (Collins et al., 2015), Italy (Giufrè et al., 2011), United States (MacNeil et al., 2011) and Canada (Public Agency of Canada, PAC, 2012). Such trends might be due to the long-term consequence of Hib vaccination. As Hib conjugate vaccination reduces pharyngeal carriage, there is a theoretical possibility that other *H. influenzae* strains such as NTHi may take its place