UNIVERSITI TEKNOLOGI MARA

CONJUGAL TRANSFER OF CO-TRIMOXAZOLE RESISTANCE GENE FROM NON-TYPEABLE *Haemophilus influenzae* TO *Haemophilus influenzae* TYPE B

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ABSTRACT

Haemophilus influenzae is one of the pathogens that reside in human nasopharynx. Amongst all the types of *H. influenzae*, type b (Hib) is the most invasive while non-typeable *H. influenzae* (NTHi) is an opportunist. Co-trimoxazole (SXT) is one of the drugs of choice to treat respiratory infections due to *H. influenzae.* However, this antibiotic has lost its potency as there are multiple reports that showed the emergence of SXT-resistant *H. influenzae* strains worldwide. A previous report in Malaysia showed that many strains of NTHi were resistant to SXT. When both strains of NTHi and Hib co-exist in the nasopharynx, there is a possibility that SXT-resistant genes from NTHi can be transferred through conjugation. Therefore, this study intents to elucidate the mechanism of transfer of SXT resistance genes from NTHi to Hib when they are in close contact. Three methods of *in-vitro* mating (filter, liquid and solid) were performed to transfer SXT-resistant genes from NTHi (strain H152) to an SXT-sensitive Hib (strain H582). Attempts were made by varying the donor-to-recipient ratio (1:1 and 1:10). However, all three mating methods used failed to produce any transconjugants. In order to understand the reason of the inability of H152 to transfer the SXT-resistant genes to H582, the DNA of both strains was sent for whole genome re-sequencing. Comparative analysis of amino acid sequences of both strains revealed that H152 contained 11 amino acids mutation in dihydrofolate reductase (DHFR) and insertion of five amino acids in dihydropteroate synthase (DHPS) that contributed to the SXT-resistance. In addition, the attachment site *attP*, the DNA region for site-specific recombination, was present only in H582 but not in H152. Type IV secretory pathway (VirB4) components required for conjugation were only present in H582 and absent in H152. As a conclusion, NTHi strain H152 used in this study was not able to transfer the SXT-resistant gene to H582 due to lack of gene components for conjugation.

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CHAPTER ONE
INTRODUCTION

1.1 Background

Antimicrobials is a great discovery in human mankind as these agents are able to combat infectious diseases, which are the leading cause of the mortality and morbidity worldwide (Aminov, 2010). However, the glory of "antibiotic era" does not last long, as the emergence and spread of antimicrobial resistant strains have been reported globally. Antimicrobial resistance is the inability of antimicrobial agents to kill or inhibit the growth of a microorganism that infects the hosts. Infections caused by bacterial strains that are resistant to antimicrobial agents are difficult to treat and the cost of the treatment is also high. The widespread of antimicrobial resistant strains are inevitable (Wise et al., 1998). This problem is usually associated with high usage of broad-spectrum antibiotics. Some bacteria develop resistance to antibiotics by various mechanisms to protect themselves against the killing action of antibiotics. This is one of the strategies for bacteria to survive in a deprived environment. Therefore, a better understanding of the mechanisms in antimicrobial resistance strains is crucial to prevent the spread and development of the resistance in bacteria.

Sulphonamide is a class of antimicrobial agents discovered through chemical application in 1932 and has a broad application against both gram-positive and gram-negative bacteria. Meanwhile, trimethoprim, a synthetic antibiotic was introduced later than sulphonamide and used to treat patient with acute urinary tract infections in 1979. In clinical application, a sulphonamide antibiotic, sulfamethoxazole is used in combination with trimethoprim to form co-trimoxazole (SXT). SXT either in a combination or as a single agent is known to be an essential drug. Both sulfamethoxazole and trimethoprim act as a bacterial folic acid inhibitor, which will inhibit DNA synthesis and further prevent the bacterial cells growth (Eliopoulos & Huovinen, 2001; Anderson et al., 2012).

SXT, which consists of one part of trimethoprim and five parts of sulfamethoxazole is used to treat a variety of bacterial infections (Wolverton, 2012). It is usually prescribed to patients with upper respiratory tract infections due to bacteria such as *Haemophilus influenzae* and *Streptococcus pneumonia*. It was also used to treat