



Biofilm forming ability of intermediate and saprophytic *Leptospira* on abiotic and biotic surfaces

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ABSTRACT

Aims: *Leptospira* spp. has the ability to develop biofilm communities and this attribute is an essential factor to leptospiral pathogenesis. This study aims to assess and quantify the biofilm forming ability of intermediate and saprophytic *Leptospira* strains.

Methodology and results: The biofilm assay was quantified on microtitre polystyrene plates (abiotic) and wood chips (*Jelutong Paya* hardwood) over a duration of 11 days. Phase contrast light microscope was used to assess the structure of the on the surface. The biofilm production on wood chips surface were approximately one times higher than on polystyrene plate surface indicating *Leptospira* strains were capable of forming higher quantity of biofilm on biotic surface compared to abiotic surface by both intermediate and saprophytic *Leptospira*. A significant difference ($p < 0.05$) exists in biofilms produced by *Leptospira* on wood surface which formed more biofilm than on polystyrene surface. The strongest biofilm producer is intermediate strain G14 with OD_{600} of 2.283 ± 0.180 and OD_{600} of 2.333 ± 0.037 , on polystyrene and wood surface, respectively. Visualisation of biofilm by phase-contrast microscopy of two representative strains correlated with the OD values and the colour intensity of stained microtitre plates and wood surfaces. The biofilm formed comprises of a three-step process are adherence (1th to 24th h), maturation (6th to 7th day) and detachment (9th to 11th day) of biofilms.

Conclusion, significance and impact of study: The contact time of intermediate pathogenic strains was faster compared to saprophytic strain, indicating the biofilm forming ability is related to the level of pathogenicity of *Leptospira* strains.

Keywords: Polystyrene, woods, biofilm formation, *Leptospira*, intermediate, saprophytic, pathogenicity

INTRODUCTION

Leptospirosis is a zoonotic disease spread by infected animals such as mice, fish and birds. It is a re-emerging disease that is caused by pathogenic *Leptospira*. It is transmitted through contact with urine, water, or soil contaminated by urine from animal reservoirs, such as rodents, dogs, and livestock (Guerra, 2009). Direct penetration of *Leptospira* sp. through the conjunctiva or surface epithelium could also cause the transmission to occur (Russ *et al.*, 2003). *Leptospira* spp. can be divided into three types which are known as saprophytic, intermediate, and pathogenic (Ristow *et al.*, 2008). In Malaysia, the Prevention and Control of Infectious Diseases Act 1988 has officially published leptospirosis as a notifiable disease in 2010.

More than 500,000 cases of severe leptospirosis occur each year, with a mortality rate 5-20% (WHO, 1999). A total of 13,176 leptospirosis cases were reported from the year 2014 until July 2015 in Malaysia, whereby 122 were death cases (Ministry of Health Malaysia,

2015). In Sarawak, a total of 163 Leptospirosis cases were reported caused by leptospirosis according to the Sarawak Weekly Epid News (March 2015). In the recent study conducted by Pui *et al.* (2015), pathogenic and intermediate *Leptospira* strains at two national parks in Sarawak were detected by PCR analysis. However, there are limited epidemiological studies of leptospirosis caused by intermediate strains of *Leptospira*.

Like other spirochaetes, *Leptospira* spp. has the ability to alter their morphology depending on the environmental conditions. The changes include the aggregation and colonization of single planktonic cells into the biofilm mode. In previous studies, bacteria have been proven to be able to exhibit biofilm in both abiotic and biotic surfaces. The potential of *Leptospira* in forming biofilms on these surfaces play an important role, not only for survival strategy but also to ensure it is successful in disease transmission and pathogenesis of these species (Iraola *et al.*, 2016). The capability of the bacteria to form biofilm on abiotic and biotic surfaces causes the increase in the persistency of these species.

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