Tropical Biomedicine 25(3): 259–261 (2008)

Research Note

Occurrence of virulent genes among environmental isolates of *Legionella pneumophila* serogroup 1 strains from various parts of peninsular Malaysia

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Received 28 July 2008; received in revised form 13 October 2008; accepted 17 October 2008

Abstract. *Legionella pneumophila* are intracellular pathogens, associated with human disease, attributed to the presence and absence of certain virulent genes. In this study, virulent gene loci (*lvh* and *rtxA* regions) associated with human disease were determined. Thirty-three cooling tower water isolates, isolated between 2004 to 2006, were analyzed for the presence of these genes by PCR method. Results showed that 19 of 33 (57.5%) of the *L. pneumophila* serogroup 1 isolates have both the genes. Six (18.2%) of the isolates have only the *lvh* gene and 2 (6.1%) of the isolates have only the *rtxA* gene. However, both genes were absent in 6 (18.2%) of the *L. pneumophila* isolates. The result of our study provides some insight into the presence of the disease causing *L. pneumophila* serogroup 1 in the environment. Molecular epidemiological studies will provide better understanding of the prevalence of the disease in Malaysia.

Legionella pneumophila are infectious agents that are ubiquitous in natural water, potable water and man made systems such as cooling water towers and spa's (Huang *et al.*, 2004). *Legionella* sp. is an intracellular pathogen which can invade and multiply in free living protozoa, making it difficult to be eradicated from water systems (Harb *et al.*, 2000). The disease in humans caused by *L. pneumophila* varies from mild respiratory illness to an acute life threatening pneumonia. Infections are usually caused by aspiration of the *Legionella* from a contaminated environmental source. There are more then 40 species of *Legionella*. However, a vast majority of Legionnaires' disease cases are attributed to *L. pneumophila* serogroup 1 (Samrakandi *et al.*, 2002).

Pathogenicity of the *L. pneumophila* serogroup 1 is related to the presence or absence of certain virulent genes within the strains. The genetic basis for virulence differences in the subgroup may affect the ability of the strains to survive in the environment and cause disease (Samrakandi *et al.*, 2002). The extensively reviewed virulent genes include type IV secretion system genes *icm/dot*, *tra1*, and *lvh* genes, the type IV pilus genes (*pilDE*) and other genes such as macrophage infectivity potentiator gene (*mip)*, the *dot/icm*-regulated pore-forming toxin gene (*rtxA*), and secretary protein encoding gene (*enhC)* (Huang *et al.*, 2004). A recent study (Samrakandi *et al.*, 2002) shows that the *lvh* and *rtxA* genes were found more frequently in *L.pneumophila* serogroup 1 strains associated with human disease. Studies on *L.pneumophila* in Malaysia were so far superficial, especially in recognizing the types of strains isolated from the cooling

towers. Therefore the aim of this study was to determine the presence or absence of the *lvh* and *rtxA* genes within the isolated *L.pneumophila* serogroup 1 strains from cooling tower.

A total of 70 cooling tower water samples from various parts of Malaysia were collected from 2005 to 2006 for the detection of *L.pneumophila*. A total of 33 out of 70 water isolates positive for *L. pneumophila* serogroup 1 were subjected to *lvh* and *rtxA* gene detection. From these, 27 isolates harboured the virulent genes, either both or one of the genes (Table 1).

Table 1: Detection of virulent genes *lvh* and *rtxA* genes in isolates from cooling tower water samples. (n=33)

Both *lvh* and *rtxA* gene	19
lvhA gene	6
rtxA gene	2
Both genes not present	6

A number of virulent genes have been described for *L. pneumophila*. These genes include type IV secretion system genes , type IV pilus genes, macrophage infectivity potentiator genes, genes encoding the *dot/icm*-regulated pore-forming toxin gene and gene encoding a secreted protein for the interaction of *L. pneumophila* with the host cells (Huang *et al.*, 2004).The *lvh* gene is categorized under the type IV secretion system genes (Segal *et al.*, 1999) meanwhile the *rtxA* gene encodes the *dot/icm*-regulated pore forming toxin gene (Zink *et al.*, 2002).The *lvh* and *rtxA* genes are found more frequently in *L. pneumophila* serogroup 1 strains associated with human diseases (Samrakandi *et al.*, 2002).

In this study, both *lvh* and *rtxA* genes were predominant in 57.6% of the cooling tower isolates. Similar observation was reported in Australia by Huang *et al.* (2004) in which 57.7% of their water isolates carried the *lvh* and *rtxA* genes. They also performed Amplified Fragment Length Polymorphism (AFLP) to subtype the *L. pneumophila* serogroup 1 isolates and have several AFLP types. Water isolates with the *lvh* and *rtxA* genes fall predominantly within the AFLP type AF1 (48.0%) and other AF types (8.7%).

It is clearly indicated that the AF1 isolates carrying both *lvh* and *rtxA* genes are widely distributed and humans have a higher chance of exposure to infection (Huang *et al.*, 2004). However, the immunofluorescence assay results for *L. pneumophila* antibody detection assay from clinical specimens, performed at the Bacteriology Unit, Institute for Medical Research, Kuala Lumpur from the year 2004 to 2006, shows a prevalence of only 1.54% (n=1816).

Low incidence pattern of the disease in Malaysia in comparison to other countries, could be due to the poor diagnosis of the disease caused by *L. pneumophila*, usually present as severe atypical pneumonia or Legionnaire's disease by the physicians. Current recommendations to treat, undiagnosed atypical pneumonia include the use of antibiotics such as erythromycin, ciprofloxacin and rifampicin (Murray, 2005) which are effective against *Legionella* and thus overlooked.

However, infection and clinical manifestation are also related to the immune status and other risk factors of the patient at the time of infection. In our study, the water isolates are limited to cooling tower water only which might also indicate that the exposure of aerosols generated from the cooling towers to humans is low.

Table 1 shows that isolates tested were found not to carry the *lvh* gene (18.1%) or *rtxA* gene (6.1%). This may be due to multiple passages on laboratory media which results in the complete loss of virulence (McDade & Shepard, 1979). Pathogenesis involves the ability to interact with host cells that allows avoidance of host defenses, survival and replication. In laboratory media, many of these bacterial factors are not needed and expressed. This phenomenon changes the genes or the genes may be lost upon passage in the laboratory (Samrakandi *et al.*, 2002).

Huang *et al.* (2006) also showed that 18.1% of the isolates neither carried the *lvh* nor the *rtxA* genes. All the isolates fall into the AF16, which is not found in clinical isolates.

Our study provided some insight on the occurrence of virulent gene in the *L.pneumophila* serogroup 1 isolates from the environment. Further studies need to be carried out to determine the virulence determinants and to epidemiologically discriminate the isolates. Currently, diagnosis of *L.pneumophila* infection from clinical specimens is not favoured since culture is tedious and can be grown only on special media. However, only identification of *L.pneumophila* serotypes through culture can be used to trace environmental sources of infection (Murray, 2005).

Acknowledgement. This work was supported by a grant from the Ministry of Health (IMR/PK/06/008). The authors would like to thank the Director General of Health of the Ministry of Health, Malaysia for permission to publish this paper. We would also like to thank all the staff of Bacteriology Unit, Institute for Medical Research for their direct or indirect contributions towards this research.

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