# Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 8 (3): 234-238
1st April, 2008

# Adherence of Virulent and Avirulent *Legionella* to Hydrocarbons

[1]M.A. Halablab and [2]A. Al-Dahlawi

The hydrophobic nature of the outermost surface of microbial cells has been implicated in their interaction with phagocytes and attachment to host cells. The interaction between seven isolates of *Legionella pneumophila*, of differing virulence and n-hexadecane and n-octane was investigated. Virulent strains had a higher affinity to hydrocarbons than avirulent strains. The hydrophobicity of strains appeared to be related to $LD_{50}$ hyperbolically and an empirical expression relating the two variables was derived. This report extends the use of microbial adherence to hydrocarbons (MATH) as a possible tool for distinguishing between pathogenic and non-pathogenic *Legionella* strains. In the context of this study, the terms hydrophobic *Legionella* and hydrophilic *Legionella* are used to indicate the affinity of the organism to hydrocarbons.

**Key words:** *Legionella pneumophila*, hydrocarbons, virulence, hydrophobicity

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Dr. M.A. Halablab
Department of Biological and Environmental Sciences,
Faculty of Science,
Beirut Arab University,
P.O. Box 11-5020, Beirut,
Lebanon

Tel: +961 1 300110
Fax: +961 1 818402

ANSInet
Asian Network for Scientific Information

[1]Department of Biological and Environmental Sciences, Faculty of Science, Beirut Arab University, Beirut, Lebanon
[2]King Abdul Aziz University, Jeddah, Saudi Arabia

## INTRODUCTION

*Legionella pneumophila*, the causative agent of Legionnaires' disease, is a facultative intracellular parasite that is capable of multiplying in human phagocytes and protozoa (Horwitz, 1988; Halablab *et al*., 1990a). The first step in phagocytic ingestion is adhesion of the invading particle to the phagocyte surface. Once attachment has taken place, a cohesive force prevents their separation despite their circulation in the blood stream. The physicochemical mechanism of this adhesion may be either a Van der Waals-type attraction (van Oss *et al*., 1983), a hydrophobic attraction and/or (more rarely) an electrostatic attraction (Nagura *et al*., 1977), or a receptor mediated bond. Albertsson (1971) introduced the use of immiscible aqueous dextran and polyethylene glycol solution in partition separation of microorganisms. Similar techniques were used (Stendahl *et al*., 1973) to demonstrate that the more hydrophobic (rough forms) of salmonellae are phagocytized more readily than smooth forms. Later, an interesting and novel approach for measuring cell surface hydrophobicity based on microbial adhesion to hydrocarbons (MATH) (Rosenberg *et al*., 1980, 1981, 1982; Rosenberg and Rosenberg, 1981) was introduced. The inherent simplicity of their technique, which involves mixing washed suspension of cells with hydrocarbons and observing their adhesion, has made it a popular assay for testing surface hydrophobicity of microorganisms (Wojnicz and Jankowski, 2007; Szabelska *et al*., 2006).

The majority of legionellae isolated from the environment are non-pathogenic but estimating their virulence in terms of their $LD_{50}$, with guinea pigs for example, is laborious and expensive. In this study, we report a novel application of the MATH assay to virulence estimation which is relatively inexpensive, rapid and may be a convenient way of assessing the pathogenicity of virulent and avirulent isolates of *Legionella*.

## MATERIALS AND METHODS

A total of seven strains (Table 1) of *Legionella pneumophila* serogroup 1 were used in this study. The Corby Av strain was isolated in our laboratory following two passages of the Corby strain ($LD_{50}$ $1\times10^{2.2}$ as estimated by aerosol infection of guinea pigs (Jepras *et al*., 1985) on Mueller-Hinton agar (Difco) supplemented with 0.025% ferric citrate and 0.025% cysteine (Sigma). This has been reported to act as a selective medium for growing avirulent *L. pneumophila* (Catrenich and Johnson, 1988). The virulence of the latter derivative was estimated to be $4.9\times10^4$, using a previously established technique (Halablab *et al*., 1990b).

Multiple sub-culture of strains was avoided by maintaining original cultures at -70°C on glass beads and inoculating onto buffered charcoal yeast extract agar supplemented with α-ketoglutarate (Edelstein, 1981) (α-BCYE) for 72 h at 37°C only once for each experiment. Cells were then harvested from the plates, washed three times in saline (1.25% NaCl) and adjusted to an optical density ($OD_{400}$ nm) of 0.8 units (final volume 3 mL).

**Math:** For the MATH assay, a modification of the method of Rosenberg and colleagues developed in 1980 was used. To the washed cells (3 mL) in acid-cleaned test tubes, 0.2 mL of either n-hexadecane or n-octane was added. The mixture was then mixed by vortexing for timed periods. After phase separation, the optical density (400 nm) of the aqueous phase was determined. Results were recorded as the percentage change in turbidity which was assumed to reflect the number of cells that partitioned into the aqueous phase.

**Light microscopy:** After vortexing the cells with hydrocarbons, a drop of n-octane-associated organisms was taken onto a glass slide and was examined under an Olympus BH2 microscope fitted with a 100X phase contrast objective.

Table 1: Characteristics of strains used in this study

| Reference strain | Monoclonal type | $LD_{50}$ | History | Source |
|---|---|---|---|---|
| 1400 (Corby) | Pontiac | $1\times10^{2.2}$ | Clinical isolate | Porton Down, UK |
| 1172 | Pontiac | $1\times10^{4.6}$ | Environmental isolate from site epidemiologically associated with disease | Porton Down, UK |
| 1018 (74/81) | Pontiac | $5\times10^4$ | Clinical isolate | Porton Down, UK |
| 1627 (Dodge) | Pontiac | $1\times10^{4.7}$ | Clinical isolate | Porton Down, UK |
| 1142 | Olda | $1\times10^{5.2}$ | Environmental isolate from site with no known association with disease | Porton Down, UK |
| 1397 | Pontiac | $>1\times10^6$ | Philadelphia strain | Porton Down, UK |
| 1400Av Corby Av‡ | Pontiac | | Corby isolate subcultured twice on Mueller-Hinton | Our laboratory |

‡ Additional details in text

## RESULTS AND DISCUSSION

Figure 1a shows the effect of vortexing time on virulent isolate (1400), an attenuated strain of the same isolate (1400 Av) and an avirulent strain (1397) and on surface hydrophilicity in n-hexadecane (a) and n-octane (b). In both hydrocarbons the hydrophilicity of the virulent strain decreased with increased vortexing time while the avirulent strain remained relatively constant. However, the avirulent isolate was at all times more hydrophilic than the virulent strain. The Corby Av variant showed intermediate attachment to the hydrocarbons used. Cell-coated hydrocarbon droplets were stable at room temperature for several days. This was confirmed by microscopic examination of the samples (Fig. 2) which related a significant number of virulent cells associated with hydrocarbon droplets. This resulted in a greater decrease in the optical density of the aqueous phase than when avirulent microorganisms were used.

Longer mixing times than that indicated in Fig. 1b resulted in fluctuating turbidity readings. This is probably due to coalescence of the hydrocarbon droplets and desorption of the bacteria into the bulk aqueous phase. More legionellae appeared to adhere to octane than to n-hexadecane, an observation which is in agreement with previous reports (Rosenberg *et al*., 1982, 1983).

It is noteworthy that attachment of microorganisms to hydrocarbons was closely related to adhesion to other substrata of interest (Rosenberg and Doyle, 1990). Several microorganisms with pathogenic properties have been shown to adhere to different hydrocarbons (Rosenberg *et al*., 1980; Magnusson and Johansson, 1977). Other studies have proposed (Ofek *et al*., 1983) that *Streptococcus pyogenes* cells are hydrophobic during adhesion and colonization and subsequently elaborate a hydrophilic capsule which protects the organism against phagocytosis. *L. pneumophila* has not been reported to colonize humans (Bridge and Edelstein, 1983). A high percentage of clinical isolates, from different sources including infected catheters and tracheal and bladder devices, have shown affinity to hydrocarbons (Boujaafar *et al*., 1990). It is, therefore, likely that virulent legionellae, which have a tendency to adhere, might behave in a similar manner and hence be more hydrophobic. Surface hydrophobicity of microbial cells influences their uptake by phagocytes; bacteria that are more hydrophobic than the phagocytes are readily phagocytozed; bacteria that are more hydrophilic than the phagocytic cells resist phagocytosis. Avirulent *L. pneumophila* have been shown to resist phagocytosis (Horwitz, 1987). Legionellae multiply in eukaryotic cells.

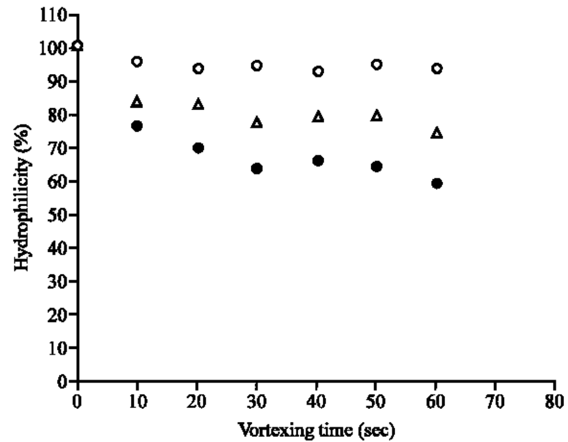


Fig. 1a: Surface hydrophobicity test, using n-hexadecane (0.2 mL), of virulent (Corby) (●), Corby Av (Δ) and avirulent strain Philadelphia-1 (1397) (○)

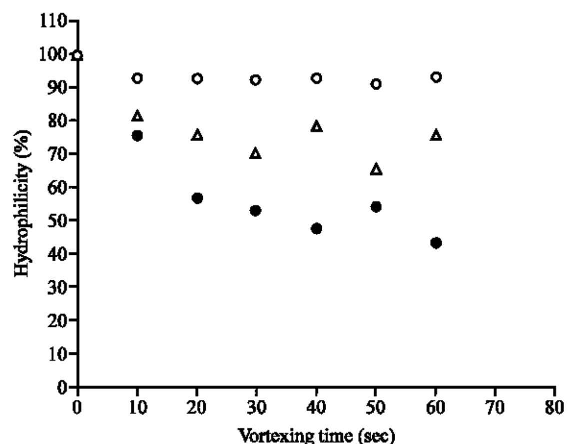


Fig. 1b: Surface hydrophobicity test, using n-octane (0.2 mL), of virulent (Corby) (●), Corby Av (Δ) and avirulent strain Philadelphia-1 (1397) (○)

Phagocyte-phagocytes-hydrophobicity interaction might be necessary for such organisms to attach and gain entry into mammalian cells and subsequently, to initiate infection. If avirulent isolates fail to attach then they will not be ingested and will ultimately be removed from the body.

Figure 3 shows the relationship between hydrophilicity after 30 sec vortexing and $LD_{50}$ as estimated by aerosol infection of guinea pigs. For the more virulent strains (lower $LD_{50}$s), there is a rapid decrease in hydrophilicity but at higher $LD_{50}$ values relatively little change occurs. The dependence of % hydrophilicity (H) on $LD_{50}$ (L) can be described in terms of a simple, empirical saturation function of the form:

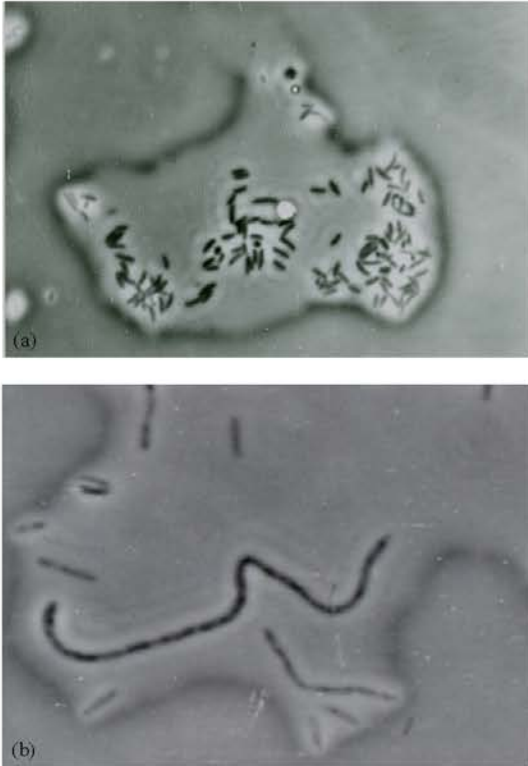


Fig. 2: Adhesion of avirulent (a) and virulent (b) *Legionella* cells to droplets of octane. Fewer avirulent cells, which were mostly filamentous, associated with the hydrocarbon. Magnification was X10000 (a) or X2250 (b)

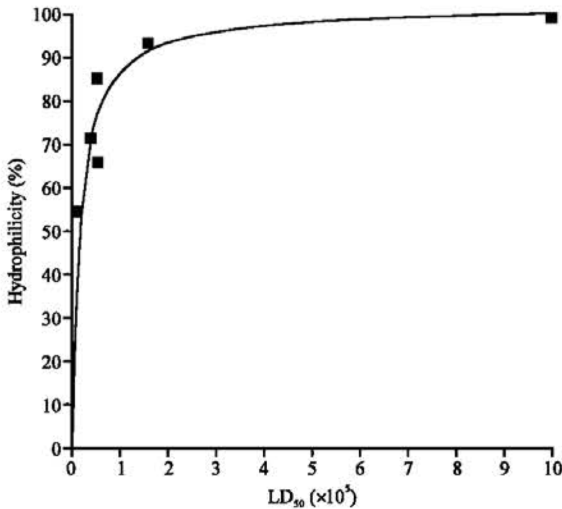


Fig. 3: Adherence of six strains of *L. pneumophila* to n-octane as a function of $LD_{50}$. Cells were vortexed with the hydrocarbon for 30 sec

$$H = \frac{H_m L}{K + L} \quad (1)$$

where, $H_m$ and k are constants. The theoretical value of $H_m$, the maximum % hydrophilicity, is 100. Using the Marquardt algorithm (Marquardt, 1963) available in the computer package Regression (Blackwell Scientific Software, Oxford, 1989) we computed a value for $H_m$ of 100.85/7% and an $LD_{50}$ for k of $1.7/0.7\times10^4$.

Rearrangement of Eq. 1 gives:

$$L = \frac{kH}{H_m - H} \quad (2)$$

This equation has the potential of forming the basis of predicting the virulence of *Legionella* strains in terms of their $LD_{50}$ values from hydrophilicity data. For such purposes it has the particular advantage that it is more discriminatory for virulent organisms and consequently might be of significant practical value.

Present results indicate the possibility that a surface component(s) of *L. pneumophila* mediates attachment to hydrocarbons. Although the MATH assay does not determine specific surface properties of microorganisms, the results were in general agreement with the so-called hydrophobicity tests reviewed by Rosenberg and Doyle (1990). In addition, organisms which do adhere in the MATH assay often tend to adhere to solid surfaces (Busscher *et al*., 1990). The lipopolysaccharide of virulent and avirulent *L. pneumophila* of the same serogroup have very similar SDS-PAGE pattern (Horwitz, 1987; Conlan *et al*., 1988). However, a better understanding of its configuration with regard to hydrophobicity is still to be determined.

## CONCLUSION

Until the surface component(s), or other factors, which mediate the MATH assay are known, the data presented in this report offer a simple and rapid system for differentiating between virulent and avirulent legionellae

## REFERENCES

Albertsson, P.A., 1971. Partition of Cells, Particles and Macromolecules. Wiley (Interscience), New York.

Boujaafar, N., J. Freney, P.J.M. Bouvet and Jeddi, 1990. Cell surface hydrophobicity of 88 clinical strains of *Acinetobacter baumannii*. Res. Microbiol., 141: 477-482.

Bridge, J.A. and P.H. Edelstein, 1983. Oropharyngeal colonization with *Legionella pneumophila*. J. Clin. Microbiol., 18 (5): 1108-1112.

Busscher, H.J., J. Sjollema and H.C. van der Mei, 1990. Relative Importance of Surface Free Energy as a Measure of Hydrophobicity in Bacterial Adhesion to Solid Surfaces. In: Microbial Cell Surface Hydrophobicity, Doyle, R.J. and M. Rosenberg (Eds.). ASM, Washington, DC.

Catrenich, C.E. and W. Johnson, 1988. Virulence conversion of *Legionella pneumophila*: A one-way phenomenon. Infect. Immun., 56 (12): 3121-3125.

Conlan, J.W., A. Williams and L.A.E. Ashworth, 1988. *In vivo* production of a tissue-destructive protease by *Legionella pneumophila* in the lungs of experimentally infected guinea pigs. J. Gen. Microbiol., 134 (2): 143-149.

Edelstein, P.H., 1981. Improved semi selective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol., 14 (3): 398-303.

Halablab, M.A., M. Bazin and L. Richards, 1990a. Estimation of *Legionella pneumophila* virulence by nitro-blue tetrazolium reduction. Lancet, 335 (8683): 240.

Halablab, M.A., L. Richards and M. Bazin, 1990b. Phagocytosis of *Legionella pneumophila*. J. Med. Microbiol., 33 (2): 75-83.

Horwitz, M.A., 1987. Characterization of avirulent mutant *Legionella pneumophila* that survive but do not multiply within human monocytes. J. Exp. Med., 166 (5): 1310-1328.

Horwitz, M.A., 1988. Phagocytosis of Intracellular Biology of *Legionella pneumophila*. In: Bacteria-Host Cell Interactions. Alen R. Liss, Inc., New York, pp: 283-302.

Jepras, R.I., R.B. Fitzgeorge and A. Baskerville, 1985. A comparison of virulence of two strains of *Legionella pneumophila* based on experimental aerosol infection of guinea-pigs. J. Hyg., 95 (1): 29-38.

Magnusson, K.E. and G. Johansson, 1977. Probing the surface of *Salmonella typhimurium* and *Salmonella minnesota* SR and R bacteria by aqueous biphasic partitioning in systems containing hydrophobic and charged polymers. FEMS. Microbiol. Lett., 2 (2): 225-228.

Marquardt, D.W., 1963. An algorithm for the least-squares estimation of nonlinear parameters. Soc. Ind. Applied Math., 11 (2): 431-435.

Nagura, H., J. Asai and K. Kojima, 1977. Studies on mechanism of phagocytosis I. Effect of electric surface-charge on phagocytic activity of macrophages for fixed red cells. Cell Struct. Function, 2 (1): 11-28.

Ofek, I., E. Whitnack and E.H. Beachey, 1983. Hydrophobic interactions of group A streptococci with hexadecane droplets. J. Bacteriol., 154 (1): 139-145.

Rosenberg, M., D. Gutnick and E. Rosenberg, 1980. Adherence of bacteria to hydrocarbons: A simple method for measuring hydrophobicity. FEMS. Microbiol. Lett., 9 (1): 29-33.

Rosenberg, M. and E. Rosenberg, 1981. Role of adherence in growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. J. Bacteriol., 148 (1): 51-57.

Rosenberg, M., A. Perry, E.A. Bayer, D. Gutnick, E. Rosenberg and I. Ofek, 1981. Adherence of *Acinetobacter calcoaceticus* RAG-1 to human epithelial cells and to hexadecane. Infect. Immun., 33 (1): 29-33.

Rosenberg, M., S. Rottem and E. Rosenberg, 1982. Cell surface hydrophobicity of smooth and rough *Proteus mirabilis* strains as determined by adherence to hydrocarbons. FEMS. Microbiol. Lett., 13 (4): 167-169.

Rosenberg, M., H. Judes and E. Weiss, 1983. Cell surface hydrophobicity of dental plaque microorganisms in situ. Infect. Immun., 42 (2): 831-834.

Rosenberg, M. and R.J. Doyle, 1990. Microbial Cell Surface Hydrophobicity: History, Measurement and Significance. In: Microbial Cell Surface Hydrophobicity, Doyle, R.J. and M. Rosenberg (Eds.). ASM Publications, Washington, DC.

Stendahl, O., C. Tagesson and L. Edebo, 1973. Partition of *Salmonella typhimurium* in a two-polymer aqueous phase system in relation to liability to phagocytosis. Infect. Immun., 8 (1): 36-41.

Szabelska, M., E. Gospodarek and E. Ciok-Pater, 2006. Influence of incubation conditions on cell surface hydrophobicity of *Candida* species fungi. Medycyna Doswiadczalna i Mikrobiologia, 58: 253-260.

van Oss, C.J., D.R. Absolom and A.W. Newmann, 1983. Interaction of phagocytes with other blood cells and with pathogenic and nonpathogenic microbes. Ann. NY. Acad. Sci., 416 (1): 332-350.

Wojnicz, D. and S. Jankowski, 2007. Effects of subinhibitory concentrations of amikacin and ciprofloxacin on the hydrophobicity and adherence to epithelial cells of uropathogenic *Escherichia coli* strains. Int. J. Antimicrob. Agents, 29 (6): 700-704.