

An in vitro evaluation of antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*

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A tick borne, multisystemic disease, Lyme borreliosis caused by the spirochete *Borrelia burgdorferi* has grown into a major public health problem during last ten years. The primary treatment for chronic Lyme disease is administration of various antibiotics. However, relapse of the disease often occurs when antibiotic treatment is discontinued. It is suggested that this resistance and reoccurrence of Lyme disease might be due to formation of different morphological forms of *Borrelia burgdorferi*. The two major known resistant morphologies are cyst and biofilm, which forms in response to stress conditions such as exposure to antibiotics.

To be able to provide novel and effective therapeutic approaches for physicians to explore the treatment options for chronically ill Lyme disease patients, we need to better understand the direct effect of antibiotics on the different morphological forms of *Borrelia burgdorferi*. In this study, we tested an in vitro susceptibility of several morphological forms of *Borrelia burgdorferi* to different antibacterial agents such as tetracyclines, hydroxychloroquine and 5-nitroimidazoles. Cell viability assays have been performed before and after the administration of the different drugs to cultures of *Borrelia burgdorferi* and different microscopic techniques such as dark field and fluorescent have been used to monitor those morphological forms of *Borrelia burgdorferi*.

Our study suggested that exposure of *Borrelia burgdorferi* cultures to concentrations greater than minimum bactericidal concentration (MBC) of doxycycline ($>25\mu\text{g/ml}$) and hydroxychloroquine (Plaquenil) ($>50\mu\text{g/ml}$) significantly reduced the spirochete population but unfortunately also increased the number of cystic forms. However, similar treatment of 5-nitroimidazoles such as metronidazole (greater than MBC as $>35\mu\text{g/ml}$) and tinidazole (greater than MBC as $>32\mu\text{g/ml}$) led to reduction of cystic forms in the culture. Furthermore, when combinations of most effective concentrations of 5-nitroimidazoles and tetracycline were tested in vitro, both cystic and spirochete forms of *Borrelia burgdorferi* were significantly eliminated. Our study suggests that *Borrelia burgdorferi* specific combination therapy for Lyme disease patients might provide treatment option with a better outcome.