

Identification of Immunoreactive Secretory Antigens from *Burkholderia cepacia* Against Monoclonal Antibody

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Keywords: *B. cepacia*; Secretome; Monoclonal antibody; Proteomic

Burkholderia cepacia is an opportunistic human pathogen associated with lung infections. Extracellular secreted proteins (the secretome) of *Burkholderia cepacia* may mediate important host-pathogen interactions known to be involved in virulence. Analysis of the secretome often provides additional information for identification of vaccine candidates and pathogenicity factors. By using two-dimensional gel electrophoresis, 285 proteins spots were detected from *B. cepacia* secreted proteins as visualised using silver staining method. Two proteins strongly reacting with monoclonal antibody raised against the *B. cepacia* secreted proteins were detected. These spots were recognised as proteins with similar molecular weight but different pI value. These antigenic proteins will be further characterised and identified.

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Biofilm Formation of *Burkholderia pseudomallei*

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Keywords: Biofilm; *Burkholderia pseudomallei*; Small Colony Variant (SCV)

Melioidosis is a potentially fatal disease caused by *Burkholderia pseudomallei* which is endemic in tropical Northern Australia and Southeast Asia. The ability to produce biofilm and evade host defense system is one of the virulence factors of gram-negative bacteria including *B. pseudomallei*. It was also found that this pathogen can produce small colony variant (SCV) phenotype. This is characterised by small colony size, slow growth on media agar and exhibition of higher biofilm formation compared to the wild type. In this study, two different modified microtiter-plate method using Luria Broth (LB) at 27°C and 37°C, and modified Vogel and Bonner Medium (MVBM), at 37°C, respectively, were used to study biofilm formation of 31 clinical isolates of *B. pseudomallei*. It was found that biofilm formation was quantitatively higher in LB medium compared to MVBM. However when comparing temperature, the biofilm production was hinger at 27°C as compared to 37°C in LB medium for all 31 isolates. SCV of isolate CTH produced the highest biofilm formation in both medium and temperatures compared to the other isolates. This study demonstrates that environmental conditions such as temperatures and medium are important component of biofilm formation by *B. pseudomallei*.

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In Vitro Bactericidal Activity of Synthetic Human Beta-Defensin 1 against Nosocomial Pathogens

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Human beta-defensin 1 (hBD-1) is a 36-amino acid cationic peptide of the innate immune system that serves as antimicrobial molecule. The objective of the study was to determine the antimicrobial properties and the bactericidal activity of synthetic hBD-1 against nosocomial pathogens belonging to 10 different bacterial species and exhibiting various antimicrobial resistance phenotypes. The native disulfide connectivity was found essential for the bactericidal activity of hBD-1, while sodium chloride concentration was reversely associated with its potency. hBD-1 exhibited high bactericidal activity against *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus* clinical isolates (the concentration of 10 µg/ml of hBD-1 was lethal for >99% of the bacterial cells). Characteristically, *A. baumannii* isolates that exhibited multidrug-resistant phenotypes were susceptible to the lowest concentrations of hBD-1 [demonstrating a virtual 90% lethal dose (vLD90) <4.5 µg/ml]. Bactericidal activity of hBD-1 was less pronounced against *E. coli*, *K. pneumoniae* and *P. mirabilis* isolates (the vLD90 varied from 5 to 50 µg/ml of hBD-1) but was significantly enhanced against isolates of these species that exhibited resistance to amoxicillin/clavulanate, aztreonam and cephalosporins (vLD90 ≤ 10 µg/ml of hBD-1). These observations give indications that the natural hBD-1 has a potential therapeutic role against bacterial pathogens and particularly against those exhibiting multidrug-resistant phenotypes.

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A Waterborne Shigellosis Outbreak in a Primary School

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Background: Shigellosis is mainly transmitted from person-to-person. Contaminated water or food can also cause large outbreaks. This study was to evaluate a waterborne outbreak caused by *Shigella sonnei* in a primary school in Tai-Chung City, November 2007.

Methods: We conducted a case-control study of students in the school on November 22, 2007. Suspect cases were defined as illness on students who had diarrhea or at least two of the following symptoms: fever, vomiting, tenesmus or abdominal pain. We used a questionnaire included

information about demographic data, illness and travel history, group gatherings, possible family clusters, hand hygiene, type of lunch, drinking water, and school cleaning assignments. Rectal swabs were collected from suspect cases, their contacts and the school cooks. We investigated the sewage system, potable and non-potable water in the school. Stool and water samples were sent for cultures and pulsed-field gel electrophoresis (PFGE).

Results: There were 275 suspect cases (attack rate = 37.1%) occurred during the 4 weeks before November 22, 2007. A total of 57 confirmed and 7 asymptomatic cases were found during the outbreak. Poor hand hygiene after toilet was associated significantly with illness (odds ratio = 1.52; 95% confidence interval: 1.03–2.23). Environmental investigation revealed that groundwater used for hand-washing in lavatories was contaminated by a leaked septic tank. One of these water samples yielded *Shigella sonnei*. The bacteria isolated from rectal swabs and from the water sample had an indistinguishable PFGE pattern.

Conclusion: It was a *Shigella* waterborne outbreak. The outbreak ended after all hand-washing facilities switched to using tap water exclusively. The outbreak did not spread to the community. Using tap water was recommended to avoid such outbreaks in schools. However, in such an outbreak, hand hygiene is still a protecting factor.

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40.060

Effect and Adverse Effect of Erythromycin Internal Use for Prevention of Whooping Cough Infection

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Background: The group infection of the whooping cough happened in the Kagawa University medical department district in May, 2007. We performed the administration of the erythromycin internal use 1,000mg a day (400-200-400) for 10 days for attached hospital personnel to prevent breakout to hospital inpatients.

Methods: Questionary survey was performed for 1,566 people to assess effect and adverse effect of erythromycin. The investigation contents were having adverse effects or not, kind of the adverse effects, compliance, developing whooping cough or not.

Results: The administration of the erythromycin was able to prevent the infection of the whooping cough. However adverse effects were different from known about it conventionally. eg) nausea, vomiting (1.2%), diarrhea (0.9%), stomachache (0.8%), and flatulence (0.8%).

Conclusion: These results indicated that the adverse effects such as the diarrhea appeared at high frequency than the things shown in the package insert. These results may lead to the improvement of the package insert of the medicine.

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40.061

Prevalence of *Bartonella spp.*, *Babesia microti*, and *Anaplasma phagocytophilum* Serologic Markers in Patients with Lyme Disease

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Background: Some pathogens, including *Bartonella sp.*, *Babesia microti* and *Anaplasma phagocytophilum*, have been identified in ticks, and cotransmission with *B. burgdorferi* has been documented. Infections, especially Babesial, can range in severity from mild, subclinical infection, to fulminate, potentially life-threatening illness. We try to assess the seroprevalence of coinfections in Lyme disease patients.

Material and Methods: Study group consisted of thirty patients with Lyme disease. Infection with *Borrelia burgdorferi* was confirmed with commercially available enzyme-linked immunosorbent assay (ELISA) and a recombinant immunoblot identifying both IgM and IgG specific antibodies. Using the indirect immunofluorescent assay (IFA) IgG antibodies against *Anaplasma phagocytophilum*, against *Bartonella sp.* (*B. henselae*, *B. quintana*) and *Babesia microti* were assessed.

Clinical manifestations and response to treatment were evaluated in the Department of Infectious Diseases, Medical University of Lublin.

Results: The IgG antibody response to *Bartonella* was found in 13 of 30 patients (43,3%), but only in 2 cases (6,7%) the titers were $\geq 1:256$, which are considered presumptive evidence of recent infection. Antibodies against *Anaplasma phagocytophilum* were found only in 2 patients (6,7%). Serologic evidences of Babesial infection were identified in 3 cases (10%). Both patients with high *Bartonella* titer were also positive in *Anaplasma phagocytophilum* and *Babesia* tests. They suffered from severe clinical symptoms of arthritis, nervous system involvement and febrile episodes.

Discussion and conclusions: Interpretation of serology results in tick-borne diseases may be difficult. High percentage of seropositivity to *Bartonella* infection and the presence of antibodies against *A. phagocytophilum* may indicate possible coinfection with other than *B. burgdorferi* agents transmitted by ticks. As symptoms of these infections may be similar to those of Lyme disease clinical diagnosis is difficult. On the other hand coinfections may explain more severe presentations. Adequate and reliable diagnosis of tick-borne diseases is of great importance due to differences in anti-infective treatment.

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Laboratory Associated Brucellosis in a Non Endemic Area

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Background and Objectives: Brucellosis, a zoonotic disease, was not endemic till 2006 among PUO cases as evident