

Fig. 1 Clinical isolate TS12 +/- AIP-3.

Fig. 2 Clinical isolate TS6 +/- AIP-1 (Ala5).

**Conclusion:** PVL positive *Staphylococcus aureus* clinical isolates express variable levels of LukF and Hla when cultured *in vitro*. Modification of *agr* activity by the addition of synthetic AIPs suggests that *agr* has a major role in regulating PVL expression in these clinical isolates. Some strains, despite having both *lukS* and *lukF* genes and producing AIP, expressed little LukF unless additional synthetic AIP was added. Upregulation of *agr* could explain the high levels of LukF expression in some isolates.

These findings reveal surprising variation in the *in vitro* expression of PVL in clinical isolates and indicate the potential for attenuating the virulence of *S.aureus*.

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47.005

**HBs mutations related with YMDD mutation induced the expression of hfgl2 gene**

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**Background:** Mutations in the highly conserved tyrosine-methionine-aspartate-aspartate (YMDD) motif are frequently associated with resistance to antiviral treatment and often followed with hepatitis flare, representing a major concern in the treatment of hepatitis B virus (HBV) infection. Previous studies showed that highly expression of hfgl2 gene is related with necrosis of hepatocytes and development of fulminant hepatitis.

**Methods:** To characterize the interaction between HBs mutations resulted by YMDD mutation and the expression of hfgl2 gene, HBs mutation expression plasmids, I195M and W196S were cotransfected with a hfgl2 promoter luciferase report construct into CHO cells and HepG2 cells respectively.

**Results:** Cotransfection of I195M or W196S with hfgl2p(-1334)LUC resulted in a significant increase in relative luciferase activity with an average increase of 3.3-fold and 3.7-fold in CHO cells, and 3.5-fold and 4.9-fold increase

in relative luciferase activity when HBs wild plasmid was cotransfected with hfgl2p(-1334)LUC in either CHO or HepG2 cells.

**Conclusion:** These results suggest that HBs mutations related with YMDD mutation induce hfgl2 promoter activity in both CHO cells and HepG2 cells. It provides new insights in the interaction between HBV mutation and host gene hfgl2 expression and the mechanism of hepatitis flare following YMDD mutation. This work was supported by NSFC No. 30972606 and National Key Basic Research Program of China 2007CB512904.

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47.006

**Enteric virus detection and identification with a universal virus discovery assay**

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**Background:** Detection and identification of known and unknown viruses can be challenging, especially for those with substantial genetic divergence, e.g. caliciviruses. To address this issue, we developed a universal virus detection assay combining virus capsid enrichment with a generic PCR. We analyzed stool and cell culture samples with our degenerate oligonucleotide primer (DOP) PCR. Caliciviruses are small, non-enveloped (+)ssRNA viruses. The family *Caliciviridae* is comprised of four genera, *Norovirus*, *Sapovirus*, *Vesivirus* and *Lagovirus*. Vesiviruses and lagoviruses infect a wide range of animal hosts; noroviruses and sapoviruses are recognized as human pathogens, causing acute gastroenteritis. Human noroviruses and sapoviruses cannot be cultured which impedes the research of these viruses considerably.

**Methods:** The first step of the assay is the physical and biochemical purification by targeted digestion of contaminating host nucleic acids followed by DOP PCR. The primer population is optimized for the detection of virus-sized genomes. Products can be identified by cloning and sequencing or by high throughput sequencing. Various DNA viruses (including HSV, VZV, SV40, AAV, EBV, parvoviruses, and hepatitis B) and RNA viruses (including HTLV-1, HTLV-2, several animal retroviruses, poliovirus, hepatitis A, human corona virus, human metapneumovirus, and influenza virus) were detected in previous studies in cell cultures and clinical samples.

**Results:** Human norovirus (stool) and feline calicivirus (vesivirus, cell culture) were identified with our universal assay. Approximately 35% of the virus genomes were obtained with a single assay. We also identified enterovirus sequences from an asymptomatic individual.

**Conclusion:** The findings presented here demonstrate the ability of the DOP-PCR assay to not only detect and identify viruses in clinical and cell culture samples but to also

provide a large portion of the sequence information with a single assay. Human diarrheal diseases cause a significant disease burden; an estimated 1.8 million deaths in children under the age of five are caused by gastroenteritis annually. Gastroenteritis is the third leading cause of death due to infection, yet, about 40% of cases are of unknown etiology. Universal detection of viruses with an assay as it was described here could lead to the detection of known yet unsuspected viruses or the discovery of novel viruses.

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47.007

#### Gene expression profiling of mouse host response to *Candida tropicalis* infection

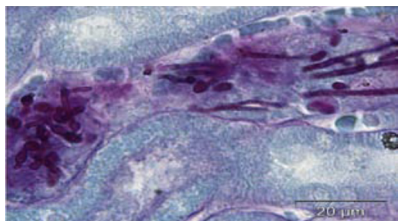
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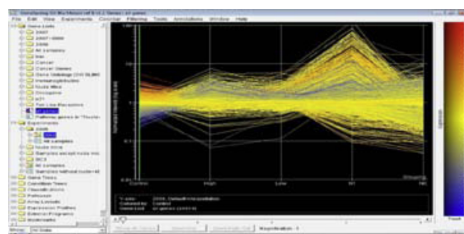
**Background:** *Candida tropicalis* is an opportunistic pathogen which can cause systemic candidiasis in immunocompromised hosts. Systemic infections caused by non-*albicans* *Candida* species, especially *C. tropicalis* has seen a rising trend. Nonetheless, studies on the global host immune and serologic responses towards the infection are lacking.

**Methods:** To further understand the effect of *Candida tropicalis* induced systemic infection on the host gene transcriptional profile, we carried out DNA microarray-based gene expression profiling of lethal infection and sublethal infection in a BALB-C mouse model. Three groups of mice comprising control (non-infected), sublethal or low infection and lethal or high-infection (inoculated with 105 and 107 *C. tropicalis* cells respectively) were sacrificed and total RNA isolated from the sera. The total RNA was reverse-transcribed and hybridized to the Illumina Mouse-Ref8 Microarray BeadChip. The gene expression level was normalized to  $\beta$ -2-microglobulin.



**Results:** The results showed that 1373 genes were differentially expressed in the lethal infection group but lower inoculum size of *Candida tropicalis* in the sublethal infection group had little effect on the host-response gene expression. For microarray data validation, multiplex RT-PCR of 19 selected genes was carried out via GenomeLab GeXP Genetic Analysis System. Confirmed upregulated genes included genes involved in host defense, pathogen recognition, signal transduction, inflammation, chemokines and cytokines, including *Ltf*, *Pglyrp1*, *Ch13l4*, syndecans, *Marco* and *Ngp*. Interestingly, we also observed differential expression of *Actb* and *Gapdh* in the lethal infection group although both are house-keeping genes normally presumed to be expressed at constant levels. From the expected functions of the genes that were upregulated in the infection groups, we speculate that *Candida tropicalis* could possibly cause

increment of erythropoiesis in the host as a compensatory mechanism for the haemolysis brought about by the metal ion-scavenging activity by *Candida tropicalis*.



**Conclusion:** Our results suggest that gene expression profiling of this mouse model may provide new insights into *Candida tropicalis* induced systemic infection particularly in finding molecular mechanisms and early biomarkers.

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47.008

#### Cytokines in experimental leptospirosis: Association with severe disease and postimmunization immune response

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**Background:** Leptospirosis shares with bacterial sepsis some clinical features, however, the leptospiral lipopolysaccharide is 10-12 times less toxic than its gram negative counterparts. Severe leptospirosis has been associated with serum levels of proinflammatory markers such as TNF- $\alpha$ , PTX3, IL6, and IL8. In addition, data from bovine whole cell antigen vaccines suggest that induction of strong Th1-type response is associated with protection. The aims of this study were to investigate: 1) gene expression of cytokines by peripheral blood mononuclear cells (PBMCs) in severe disease; and 2) gene expression of cytokines in PBMCs after immunization by whole cell vaccine and homologous challenge.

**Methods:** Gene expression of IL2, IL4, TNF- $\alpha$ , and IFN- $\gamma$  by Real Time PCR. The virulent strain used in the study was *L. interrogans* serovar Copenhageni strain Cop 4.14. To evaluate gene expression in severe disease, 25 hamsters were infected by 250 leptospores (5x lethal dose 50%) and compared to 4 uninfected controls. Hamsters immunized by whole cell vaccine and controls were evaluated at 8 time points (n=3 in each group) from 0h to 21 days.

**Results:** All infected hamsters developed lethal disease with typical target organ pathology. Gene expression was higher for all cytokines in infected animals at moribund state (7-8 days after infection) when compared to controls. The difference was statistically significant for IFN- $\gamma$  (p=0.01). Cytokines were not associated with bacterial quantification in tissue or specific target organ lesions. Immunized hamsters survived and expressed higher levels of TNF- $\alpha$  on the eighth day (145 vs 19) and IFN- $\gamma$  on the third day after infection on the third day (32 vs 0.5) after challenge, when compared to the control expression of HPRT.