substitutions/site/month). Following seroconversion, there was a striking increase in viral diversity. Most seroconverters had viral variants that showed evidence of positive selection, which was seen mainly postseroconversion. Conclusions: The high viral diversity before a reduction in HBV DNA and before HBeAg seroconversion, could either be related to occurrence of stochastic mutations that lead to a break in immune tolerance, or increased immune reactivity that drives escape mutations. In a follow-up study, we examined the long term evolution of HBV quasispecies and whether the increase in viral diversity could be confirmed. Furthermore, we sought to determine if this increase in viral diversity could result in creation of new CpG motifs, which could stimulate Toll Receptor 9. TLR9 is one member of a family of highly conserved molecular pattern recognition receptors-toll like receptors, and is stimulated by unmethylated CpG dinucleotides resulting in activation of the innate immune response and consequently adaptive immune response. In this study, we analyzed if there were any CpG sites in the HBV genome of chronic hepatitis B patients and their relationship with HBeAg seroconversion, using Eight HBeAg seroconverters followed up 6–10 years before seroconversion were included. Serum samples of 5 to 9 time points before HBeAg seroconversion were used in the study. Seven non-seroconverters with matching follow up period and stored serum samples were involved as controls. An identical methodology was used to evaluate 20 clones per serum sample and analysed using the same methodology as previously. HBV CpG status was analyzed with the sequence alignments.

**Results:** The HBV quasispecies viral diversity gradually increased from $1.4 \times 10^{-3}$ to $1.2 \times 10^{-2}$ within 9 years before HBeAg seroconversion. Positive selection was detected in 5 of 8 seroconverters in the study period. New CpG motifs also emerged before HBeAg seroconversion. The increase of viral diversity was closely correlated with the reduction of HBV DNA, HBeAg levels and increase in frequency of precore stop codon mutation.

**Conclusion:** The gradual increase of HBV quasispecies over time before HBeAg seroconversion could be related to accumulation of stochastic mutations which result in occurrence of new CpG motifs that activated the innate immune response and break the immune tolerance leading to HBeAg seroconversion.

**CS1-03 Immunological Effects of Type I Interferons in Hepatitis B Revisited: How Does Interferon Work against HBV?**

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Type I interferons have been used for almost 25 years to treat hepatitis virus infections. While HCV infection can be cured in 50-80% of cases with IFN-based treatment regimens, the goal of IFN-therapy of HBV infection is to induce an immune control which is either associated with HBe seroconversion or even loss of HBSAg. Obvious advantages of IFN therapies of chronic hepatitis B are finite treatment durations, the lack of resistance development and higher off-treatment sustained response rates as compared to HBV polymerase inhibitors. However, IFN alpha may induce various side effects and the direct antiviral potency is much weaker as compared to the recently approved nucleoside and nucleotide analogues. The detailed mode of action of IFN alpha in the treatment of chronic hepatitis B is poorly defined. Different effect of IFNAs have been proposed: (i) IFNAs directly inhibits HBV replication which has been confirmed by in vitro models of HBV replication. (ii) IFNAs may display various effects on cells of the adaptive immune system including dendritic cells and T cells. Some but not all studies suggest an impairment of dendritic cells in chronic hepatitis B and IFNAs may enhance antigen presentation, cytokine production and maturation of dendritic cells. If IFNAs treatment is associated with a reduction of the in HBV infection increased frequency of regulatory T cells (Tregs) has not been studied in detail yet. Impaired CD8+ T cell function is associated with a higher expression of PD-1 expression and blocking PD1-signalling can restore T cell activity. It is generally believed that T cell function should be increased by IFNAs, however, IFNAs has also antiproliferative capacities and may therefore impair T cell proliferation. (iii) IFNAs is believed to enhance NK cell functions. We gained evidence that IFNAs increases the cytotoxic activity of NK cells via various mechanisms including upregulation of certain molecules such as TRAIL. (iv) The potential effects of IFNAs on humoral immunity against HBV are poorly defined but several studies have shown already that B cell priming and BCR crosslinking is impaired by IFNAs. (v) Toll-like receptor-2 expression is reduced in HBeAg-positive hepatitis B. Preliminary data suggest that IFNAs may increase TLR 2 expression.

Overall, IFNAs treatment is associated with a reduced virion productivity. To what extent this due to direct intracellular mechanisms or depletion of HBV-infected cells remains to be determined. Future studies will have to define in more detail which patients are good candidates of IFNAs treatment and which patients may benefit from combination therapies with HBV polymerase inhibitors.

**CS1-04 Gender and HBV - Interaction between Human Sex Hormones and HBV Transcription/Replication**

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One interesting outcome of chronic hepatitis B virus infection has been a more common development of end-stage liver diseases in males than females. This gender disparity is more striking in HBV-related liver cancer, in which male patients usually outnumber females to 5-7 folds. The hypothesis is proposed either males more vulnerable to HBV-carcinogenesis or females more protective against this process, or both. The hypothesis has been supported by epidemiological investigations in which higher androgen levels are correlated with more risk of HCC in male carriers, and use of estrogen supplements reduces HCC in female carriers. Now we present the results how human sex hormones axis interacts with HBV. It was found that androgen receptor could bind directly to HBV genome, via enhancer I, to augment HBV transcription and replication. Besides, androgen receptor can cooperate with HBV X protein to enhance down-stream cancer-promoting pathway. In contrast, there are data indicating female estrogen receptor able to down regulate HBV gene transcription, maybe via a remodeling of viral genome. The addition of estrogen to male HBV transgenic mice could reduce HBV titers. These results may explain the mechanisms for a male susceptibility to HBV-related end-stage liver diseases and might point out directions to anti-viral targets.

**CS1-05 Immune Evasion and the Liver**

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**Concurrent Session 2 – Gram-Positive Bacterial Infections and Resistance**

**CS2-01 Clostridium difficile Infections**

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Currently, Clostridium difficile rivals methicillin-resistant
Streptococcus suis (MRSA) as the most common organism to cause healthcare-associated infections in the United States. Incidence of *C. difficile* infection (CDI) has also increased in Canada, Europe, and other parts of the world. CDI is associated with increased lengths of hospital stay, medical costs, morbidity, and mortality (attributable mortality rate of 6.9% at 30 days after diagnosis and 16.7% at 1 year) among hospitalized patients. Nearly every antibiotic along with gastric acid suppression has been associated with CDI. Among these, cephalosporins, ampicillin, and clindamycin remain important predisposing agents. Fluoroquinolones, previously infrequently associated with CDI, have been found to be one of the primary predisposing antimicrobials in recent studies. A positive result of a test for toxigenic *C. difficile* and/or its toxins in a patient with diarrhea is considered to be diagnostic for CDI. Most reports of increases in the incidence and severity of CDI have been associated with the BI/NAP1/027 strain of *C. difficile*. This strain produces more toxins A and B in vitro than do many other strains of *C. difficile*, produces binary toxin, and is highly resistant to fluoroquinolones.

Metronidazole and vancomycin remain the primary options for the treatment of CDI. Several studies suggest that rates of response to treatment of CDI with metronidazole are declining and statistically superior rates of response to vancomycin treatment for severe disease with metronidazole treatment. Two antimicrobials (nitazoxanide and rifaximin) available in the United States have been used successfully for CDI treatment but lack United States Food and Drug Administration approval for this indication. Experimental treatments currently in clinical development include a toxin-binding polymer (talevamex), two poorly absorbed antimicrobials (OPT-80 [difimicin] and ramoplanin), monoclonal antibodies, and a *C. difficile* vaccine. General strategies to prevent and reduce the risk of CDI include adherence of antimicrobial usage restriction and stewardship guidelines, preventing the patient from being exposed to *C. difficile* (disinfection and barrier precautions), and meticulous hand hygiene, and probable implementation of probiotics.

**CS2-02 Streptococcus suis Infections in Humans**

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*Streptococcus suis* is a Gram-positive coccus, causing meningitis, septicemia, pneumonia, and purulent arthritis in pigs. Human infection is acquired through exposure to colonized pigs or through direct contact, e.g. through skin lesions during butchering, but other routes of infection, such as gastro-intestinal, cannot be excluded. In the past few years the number of reported *S. suis* infections in humans has increased significantly, with most cases originating in Southeast Asia where there exists a high density of pig farming. Increased awareness, improved diagnostics and the occurrence of outbreaks have contributed to this increase. Meningitis and sepsis are the most common clinical manifestation of human *S. suis* infection. In Vietnam, *S. suis* is the most common cause of acute bacterial meningitis in adults. Infections in children are extremely rare. Meningitis is frequently complicated by sensorineural hearing loss. Adjutant treatment with dexamethasone is associated with protection against the hearing loss in *S. suis* meningitis. *S. suis* is sensitive to penicillin, although in very rare cases penicillin resistance has been reported, but high rates of resistance to tetracycline and erythromycin have been reported from human and pig isolates worldwide.

Human infections are almost always caused by *S. suis* serotype 2 (determined by the capsule polysaccharide), whilst infection in pigs is caused by a number of the 35 known serotypes. Molecular typing suggests that a very limited number of clones is responsible for human infection. Whole genome sequencing of *S. suis* serotype 2 strains indicates that strains of the same clonal complex are highly related and that variation predominantly occurs through horizontal transfer of mobile genetic elements. Increased awareness from both clinicians and microbiologists is needed to fully appreciate the importance of *S. suis* as a human pathogen.

**CS2-03 VanA Type Vancomycin Resistant Enterococci in China**

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Group A streptococci (GAS) are one of the most common human pathogens that cause both invasive and noninvasive infections. GAS associated diseases are more common in children than adults and clinical presentation ranges from pharyngitis and impetigo to invasive infections particularly acute rheumatic fever and acute glomerulonephritis. The *M* protein is an important virulence determinant in GAS which is determined by the emm gene. Currently, more than 170 emm types and 750 emm subtypes of group A streptococci have been described. Studies of GAS emm typing distribution performed worldwide, have shown a differing distribution of emm types in different countries and regions. SAGs are thought to contribute to the pathogenesis of severe GAS infections by virtue of their potent immunostimulatory activity and the distribution of profiles of genes encoding SAGs has been used as an additional epidemiological tool to explore the genomic heterogeneity and possible correlation between toxin gene content and disease type. Since the late 1990s, resistance to macrolides has been increasingly detected in *S. pyogenes* in several European countries and other parts of the world such as Korea. There are two primary phenotypes of macrolide resistance in *S. pyogenes*: the M phenotype mediated by the mef genes and the MLSB phenotype mediated by the ermB genes. The latter phenotype can be constitutive, generally mediated by the ermB gene, or inducible, generally mediated by the ermA subclass TR (ermA) gene. Many studies have presented evidence that the phenotypes and R genes of *S. pyogenes* have changed over time. We analyzed the emm type and detected the susceptibilities of antibiotics to these strains and their phenotype of macrolide resistance. We also investigated the virulence factors including the superantigens of the strains. Here, we will report that there were high resistance rates against macrolides in GAS isolates collected from Chinese pharyngitis and scarlet fever patients and that the R-gene was mainly the ermB gene. We also found that the pattern of the emm-type distribution of GAS strains from Chinese pediatric patients changed with time and there is a change in the antimicrobial susceptibility and distribution of the R-gene in a large collection of *S. pyogenes* clinical isolates from pediatric patients during the periods 1993 to 1994 and 2005 to 2008, and to establish its association with the emm-type shift.

**CS2-04 VanA Type Vancomycin Resistant Enterococci in China**

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Most of the vanA type VRE in China were *E. faecium*. The resistance rates of the vanA type vancomycin resistant enterococci isolates to erythromycin, levofloxacin, ampicillin and rifampin were 96.7%, 91.7%, 88.3% and 80.8%. All VRE isolates were susceptible to linezolid. Multilocus sequence typing (MLST) identified most of the VRE isolates belonged to clonal complexes CC17. Vancomycin resistance of vanA type *E. faecium* isolates was transferred at a frequency of 7.0 × 10^-1 to 2.3 × 10^-2 between *E. faecium* strains during filter mating. Two of the plasmids, pZB18 (67 kbp) and pZB22 (200 kbp), were highly conjugative and were able to transfer at high frequencies of around 10^-4 and