

Conclusion: Demonstrating the association between the presence of oval cells and the progression of liver diseases could provide an useful marker for understanding the correlation between the presence of oval cells and progression of disease and creation of future treatment.

OL-006 Efficacy, tolerability and safety of personalized low-dose IFN treatment in patients with HCV-related liver cirrhosis and severe complications

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Object: To observe the efficacy, tolerability and safety of personalized low-dose IFN treatment in patients with HCV-related liver cirrhosis and severe complications.

Method: Personalized low-dose IFN treatment was performed in 61 patients. Less than 3 million units of personalized low-dose natural IFN-alpha was administered QOD intramuscularly or less than 50 µg Peg-IFN alpha-2b QW intrasubcutaneously, which depended on patients tolerability, and plus Ribavirin 600 mg/day. The course of treatment was at least 24 weeks. Some patients received more than 2 years IFN maintenance therapy.

Results: Twenty of the 62 patients showed a rapid virological response in 4 weeks treatment (32.25%). Twenty eight of 62 patients showed a complete early virological response in 12 weeks treatment (45.16%). Thirty four of 62 patients showed HCV RNA undetectable in 24 weeks IFN therapy (54.83%). ALT levels normalized (about 40 IU/L) at the end of 24 weeks therapy. 11 of 25 patients had a sustained virological response who were given more than 2 years Maintenance Therapy (44%). Definitive discontinuation of therapy was necessary in 7 patients (11.29 %) because of side effects.

Conclusion: Personalized low-dose IFN and ribavirin combination therapy was useful and safe in some patients with HCV-related liver cirrhosis and severe complications for whom standard-dose interferon and ribavirin combination therapy was difficult.

Free Paper Presentation 2 – Bacterial Infections/Antibiotics I

OL-007 Carbapenems resistance in Gram-negative bacilli isolates in an intensive care unit

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Objective: to determine resistance of *Ps. aeruginosa*, *A. baumannii* and *K. pneumoniae* as prevalent nosocomial agents to commonly used antibiotics including imipenem, meropenem and ertapenem.

Methods: Identification of microorganisms and susceptibility test was performed with the Vitek 2 (BioMerieux®, France) and the susceptibility disc diffusion method according to CLSI directions. For *Klebsiella spp.* extended-spectrum Beta-lactamases (ESBLs) production was confirmed by double-disc test. To screen for metallo-β-lactamase production (MBL), a synergy test using an imipenem and EDTA-containing discs was employed. Quality control was ensured by keeping weekly records of disk diffusion *Ps. aeruginosa* (ATCC 27853). MIC values for carbapenems were determined by the E-test (AB Biodisk, Solona, Sweden) as recommended by manufacture. *K. pneumoniae* ATCC 70603 was used as a positive ESBLs strain.

Results: Information was available on antibiotic susceptibility of 1044 gram-negative bacteria, of which the most common

were *A. baumannii* 414, *Ps. aeruginosa* 328, *K. pneumoniae* 169. No duplicate isolates from the same patients were included. All microorganisms were isolated from tracheal tube aspirates, urine, wound, blood and other sterile body fluids. The resistance rates (%) of *A. baumannii*, *Ps. aeruginosa*, and *K. pneumoniae* were: imipenem 76/61/67, meropenem 68/52/65, ertapenem 77/65/68, amikacin 88/48/40, piperacillin/tazobactam 82/35/67, ceftazidime 100/68/65, ciprofloxacin 92/64/15, aztreonam 100/84/72. Of 169 isolates of *K. pneumoniae* 92 (54,43%) were ESBLs.

Conclusions: The most isolates of *A. baumannii* were multi-drug resistant. The majority of isolates were resistant to 5 or more antibiotics tested and some strains were defined by resistance to all antimicrobial agents except colistin. The resistance to carbapenems rose dramatically.

OL-008 Molecular characterization of extended-spectrum β-lactamases and AmpC enzymes in Enterobacteriaceae in Beijing, China

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Objectives: A study was conducted to evaluate the molecular characterization of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC enzymes in *Enterobacteriaceae* in Beijing, China.

Methods: Production of ESBLs and plasmid-mediated AmpC among 240 non-duplicate *Enterobacteriaceae* isolates was screened by the phenotypic methods and the molecular methods. The epidemiological relationship of the isolates was studied by random amplified polymorphic DNA (RAPD) analysis.

Results: CTX-M type ESBLs were the most prevalent ESBLs. Three *E. coli* isolates simultaneously harbored *bla*_{CTX-M-3} and *bla*_{CTX-M-9} genes. SHV-12 was the most prevalent SHV-type ESBL. SHV-2a, SHV-2 and SHV-5 ESBLs, and SHV-27 and SHV-44 non-ESBLs were detected. Two *Klebsiella pneumoniae* isolates expressed a novel ESBL, SHV-43a, which had one substitution (Leu35Gln) compared with SHV-43. DHA-1 was the most prevalent plasmid-mediated AmpC enzyme, found mainly in *K. pneumoniae* (n=11). We also identified the plasmid-mediated CMY-2 enzyme in two *E. coli* isolates. RAPD analysis revealed that 53 CTX-M-13- and 7 CTX-M-3-producing *E. cloacae* isolates recovered from a single hospital exhibited a high similarity of RAPD patterns, indicating clone-related spread.

Conclusions: This survey indicates the high frequencies of CTX-M-9/3 ESBLs and plasmid-mediated DHA-1 in China, reports the first emergence of DHA-1-producing *E. cloacae* and interhospital epidemic CTX-M-13/3-producing *E. cloacae*.

OL-009 Virulence factors determination and molecular characterisation of Malaysian *Vibrio cholerae*

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Objective: To determine the virulence profiles of Malaysian *Vibrio cholerae* and to investigate the relatedness of the strains using molecular typing methods.

Methods: 43 *V. cholerae* were isolated from clinical and environmental sources. Strains isolated were serogrouped and PCR were carried out for determination of the virulence genes harbored in each strain. The strains were further characterized using molecular subtyping methods such as RAPD, ERIC, REP-PCR and PFGE fingerprinting to investigate the relatedness among the strains.

Results: Twenty-three O1, one O139 and 19 non-O1/nonO139 strains were isolated. All but one O1 strains harbored virulence genes such as *ctxA*, *zot*, *rtxA*, *rstR*, *toxT*, *toxR*, *tcpA*, *tcpI*, and

hlyA. The O1 strains mentioned was isolated from clinical sample and negative for *ctxA*, *tcpA*, *tcpI*. *TcpI* gene was detected in 2 non-O1/non-O139 strains with the absence of *tcpA*. A combination of molecular fingerprinting method had distinguished the strains into 43 profiles with F value = 0.59 -0.95. The O1 strain mentioned was clustered in-between toxigenic O1/O139 and non-toxigenic, non-O1/non-O139 strains. As for the 2 non-O1/non-O139 strains, one of them was related with O1 toxigenic strains while another was genetically distinct from all strains.

Conclusion: The Malaysian strains isolated in this study were very diverse. The results also revealed the virulence potential of non-toxigenic, non-O1/non-O139 strains as the close relationship between the strains and O1/O139 toxigenic strains were developed and proved by the virulence profile.

OL-010 High macrolide resistance in streptococcus pyogenes strains isolated from children with pharyngitis in China

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Objectives: To assess the macrolide resistance, phenotype, and genotypic characterization of *Streptococcus pyogenes* isolated from Chinese children with pharyngitis.

Methods: Minimal inhibitory concentration with nine antibiotics was determined on 188 isolates of *S. pyogenes* collected from outpatients with pharyngitis in four children's hospitals in different regions of China in 2007. Minimal inhibitory concentrations (MICs) of penicillin, chloramphenicol, cefradine, levofloxacin, macrolide (erythromycin, clarithromycin, azithromycin,), clindamycin, and tetracycline were determined by the microdilution method. The macrolide resistant phenotypes of isolates were determined through a double-disk. The resistant genes (*mefA*, *ermB*, and *ermA*) were amplified by polymerase chain reaction (PCR).

Results: Over 95% were resistant to macrolides, while 92.0% were resistant to tetracycline. We also found that all isolates were sensitive to penicillin, chloramphenicol, cefradine, and levofloxacin. Among the 173 erythromycin resistant strains, 171 (98.8%) were assigned to the cMLS phenotype, while the remaining 2 (1.2%) were assigned to the iMLS phenotype. Among the 171 cMLS isolates, 168 isolates (98.2%) had the *ermB* gene accounting for 98.2%. Meanwhile, 2 iMLS isolates had the *ermA* gene. Macrolides were highly resistant to *ermB* positive strains (MIC₉₀ > 256 µg/ml). Neither the M-phenotype nor the *mefA* gene was detected. Meanwhile, our studies of multiple centers showed that consumption of macrolides from 2000 to 2006 was very high. **Conclusion:** The *ermB* gene code is the main resistance mechanism against macrolides in *S. pyogenes*. The high rate of macrolide resistance to *S. pyogenes* was observed, which may be correlated with the overuse of antibiotics in China.

OL-011 Mycobacterium tuberculosis L-form and RIF-dependent strains in clinical specimens

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Background: The increasing emergence of multiple-drug-resis-

tant (MDR), extensively drug-resistant (XDR), and RIF-dependent MDR-TB, emphasizes the need to investigate the mechanism of drug resistance further. *M. tuberculosis* L-form is insensitive to multiple drugs, and is often ignored in the clinic. In this study, L-form and RIF-dependent *M. tuberculosis* were investigated in clinical specimens at Lanzhou Pulmonary Hospital, and their characteristics were analyzed preliminarily.

Methods: Clinical specimens were inoculated in BBL-MGIT Middlebrook 7H9 liquid and 92-3 TB-L liquid medium without RIF, and Lowenstein-Jensen, 7H9 semi-solid, 7H9 hypertonic semi-solid medium with or without RIF. The L-form and the RIF-dependent *M. tuberculosis* were subjected to acid-fast staining. Furthermore, the *rpoB* gene from RIF-dependent and the L-form *M. tuberculosis* strains was sequenced.

Result: In 142 clinical samples, *M. tuberculosis* L-form bacteria were detected in 13 cases. The average age was 57 years old. Most of them had the history of tuberculosis or old lesions by X-ray. RIF-dependent *M. tuberculosis* was detected in 5 of 142 cases. Sequencing of the *rpoB* gene revealed mutations in codon 306 (GAT→GGT, Gly→Asp), 531 (TTG→TCG, Ser→Leu), 581 (ACT→ACC, Thr→Thr), 584 (TCC→TTC, Phe→Ser), and 832 (GCC→GCT, Ala→Ala).

Conclusion: RIF-dependent and L-form of *M. tuberculosis* are present in clinical specimens. *M. tuberculosis* L-forms mostly exist in the elderly which have a history of tuberculosis. The RIF-dependent *M. tuberculosis* strains have multiple *rpoB* mutations. The relationship of L-form to MDR-TB and RIF-dependent TB needs to be further investigated.

OL-012 Carriage of the respiratory tract bacterial pathogens in children with latent tuberculosis

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Background: Despite of the results in treatment of tuberculosis, bacterial infections are the most often complications in the patients with latent tuberculosis.

Aim: was to characterize the carriage of bacterial respiratory tract pathogens in children with latent tuberculosis and to compare these isolates with isolates gained from healthy carriers.

Methods: The isolates of *H. influenzae* (n=32), *S. pneumoniae* (n=25), etc (n=23) were isolated from lab material of 80 patients by the quantitative sputum culture method (greater than or equal to 10⁷/ml) in Far Eastern Pediatrics National Tuberculosis Center (control group (11 strains of *H. influenzae* and 17 strains of *S. pneumoniae*) was isolated from 40 healthy carrier children). Antimicrobial resistance was checked by disk-diffusion method and MIC. Multilocus sequence typing (MLST) was performed with five loci such as *adk*, *fucK*, *mdh*, *pgi*, *recA*.

Results: *H. influenzae* was the most frequent microorganism in nasopharyngeal tract in children with latent tuberculosis (40%). These strains were resistant to rifampicin 22,5%, penicillin 40%, erythromycin 82,5%, co-trimoxazole 59,37%. The antimicrobial resistance pattern in healthy children was characterized as having less resistance to penicillin (1 strain), and there were no resistant strains to rifampicine. *S. pneumoniae* isolated in children with tuberculosis was resistant to rifampicin in 2 cases from 25 strains (8%), and in 36% to macrolides. MLST showed that there were 6 clonal complexes in *H. influenzae* isolates.

Conclusions: Our study confirmed the significance of study of bacterial respiratory tract carriage and need to be continued for to development preventive regimens for such group as children with low immunity.