152. Microbiological advances in the diagnosis of tuberculosis

P1405

Comparison of molecular and immunological methods for a rapid diagnosis of smear-negative tuberculosis

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Background: A rapid diagnosis or exclusion of pulmonary tuberculosis (pTB) in pTB suspects with negative acid-fast-bacilli (AFB) sputum smears is at times still challenging in clinical practice.
Methods: We compared in retrospect results of Xpert MTB/Rif (single sputum or BAL specimen) nucleic acid amplification with a *Mycobacterium tuberculosis*-specific bronchoalveolar-lavage (BAL) Elispot in suspects of pTB with negative AFB sputum smears at a TB referral center in Germany.
Results: In 96 suspects of pTB with negative AFB sputum smears admitted from 04/2010-10/2011 in our clinic, pTB was confirmed by culture in 10 cases and clinically suspected in 9 cases. Sensitivity, specificity, positive and negative likelihood ratio were 60%, 97%, 30, 0.41 for culture confirmed cases and 42.1%, 97%, 21.1, 0.59 for all TB patients for the Xpert MTB/Rif and 80%, 62.6%, 2.1, 0.32 for culture confirmed cases and 89.4%, 62.6%, 2.4, 0.17 for all TB patients for the *M. tuberculosis* specific BAL-Elispot. BAL-Elispot identified 10 out of 11 patients with pTB (including 3 out of 4 patients with culture confirmed TB) with a negative Xpert MTB/Rif test.
Conclusion: A positive result of a Xpert MTB/Rif test on a sputum or BAL specimen has a very high likelihood for the diagnosis of active TB, however the sensitivity is insufficient to rule out pTB with a negative test result. BAL-Elispot identifies the majority of TB cases in suspects of pTB with negative AFB sputum smears and negative Xpert MTB/Rif test but the specificity of the BAL-Elispot is suboptimal for a confirmed diagnosis.

P1406

Diagnostic role of micro-MGIT culture of BAL samples in sputum smear-negative pulmonary tuberculosis

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Introduction: In view of the diagnostic difficulties associated with sputum- negative pulmonary TB (PTB), we aimed at exploring if bronchoalveolar lavage (BAL) samples can be subjected to smear- microscopy and rapid mycobacterial culture (by Mycobacterial Growth Indicator Tube method) to achieve improved diagnosis of this condition.
Methods: Patients presenting with clinico-radiological features suggestive of pulmonary tuberculosis and whose sputum smears were negative for acid- fast bacilli (AFB) or who could not expectorate sputum were prospectively enrolled in this study. BAL samples collected from them were subjected to smear- microscopy for AFB and micro-MGIT culture. BAL samples were also inoculated on Lowenstein-Jensen (LJ) slants.
Results: A total of 105 patients (74 males) were recruited in the study, with a mean (±SD) age of 51 (± 15) years. The diagnosis of PTB was made in 52 patients on the basis of clinico- radiological presentation, with or without microbiological confirmation. Thirty- four patients (65.4%) had microbiologically confirmed PTB. Of them, AFB was detected in 12 BAL samples, while culture- positivity was noted in 24 and 27 patients by the LJ and MGIT methods respectively. Inter- test agreement between the LJ and MGIT methods was found to be significant (κ= 0.655; p= <0.001). However, the mean time to positivity was significantly lower for the MGIT method than for the LJ method (p= <0.001).
Conclusion: Examination of BAL samples by smear- microscopy and micro-MGIT culture can, therefore, provide a rapid and definitive diagnosis of PTB in sputum-negative patients.

P1407

Effective variable-number tandem repeats loci for discrimination of *Mycobacterium tuberculosis* isolated from Korea

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Introduction: The variable-number tandem repeats (VNTR) typing is a promising method to discriminate *M. tuberculosis* isolates in molecular epidemiology.
Objectives: The purpose of this study was to evaluate already known VNTR loci and to select best combination of VNTR loci set for discrimination Korean TB strains.
Methods: The 307 clinical isolates collected from throughout Korea were genotyped using IS*6110* RFLP, and analyzed the number of VNTR copies for the 32 VNTR loci including Supply-15, Supply-24 and JATA-15(except QUB-18); MIRU-02, -04, -10, -16, -20, -23, -24, -26, -27, -31, -39,- 40, ETR-A, -B, -C, QUB-11a, -11b, -15, -26, Mtub-04, -21, -24, -29, -30, -34, -39, VNTR-2372, -3232, -3336, -3820, -4120, -4156. And then, allelic diversity (*h*) and the Hunter-Gaston discriminatory index (HGDI) were calculated to evaluate discriminatory power of locus and combination of VNTR loci.
Results: As a results of analysis of the 32 VNTR loci, we found that 12 loci (MIRU-26, -31, QUB-11a, -11b, -26, Mtub-04, -21, VNTR-3232, -3336, -3820, -4120, -4156) showed high discrimination power (each *h* values of them was over 0.6). This new 12-locus combination for Korea TB strains was designated as a KIT-12. The discriminatory index (HGDI) of IS*6110* RFLP and VNTRs of Supply-15, Supply-24, JATA-15, and *KIT-12* was 0.9992, 0.9980, 0.9987, 0.9992, and *0.9997* respectively. Also the percentage of clustered cases was 16.6%(51/307), 21.8%(67/307), 16.9%(52/307), 14.3%(44/307), *6.8%(21/307)* respectively.
Conclusions: The newly proposed VNTR typing system, KIT-12 loci can be effective tool for *M. tuberculosis* genotyping in Korea where the Beijing strains are predominant.

P1408

Evaluation of real time polymerase chain reaction, adenosine deaminase and interferon gamma in tubercular pleural effusions

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Introduction: Pleural effusions are a common manifestation of tuberculosis. Real Time Polymerase Chain Reaction (RT-PCR), Adenosine Deaminase (ADA) and Interferon gamma release assay (INF) provide faster results than Ziehl Neelsen (ZN) staining and Culture on LJ medium. RT-PCR is superior to conventional PCR in sensitivity, specificity with lower contamination and reduction in time to result.
Aims and Objectives: To evaluate the sensitivity of RT-PCR, ADA and INF in cases of pleural effusions due to tuberculosis.
Methods: RT-PCR was performed in 168 patients of tubercular pleural effusions. All patients had positive Mantoux test with high protein and lymphocyte predominance in effusion.
RT-PCR was performed by detecting amplification reaction for the insert element IS6110 of the *Mycobacterium tuberculosis* complex (Biotub-QT, Biotools Labs, Spain) using a real-time centrifugal amplifcation system (Rotor-Gene 3000, Corbett Research, Australia).
ADA was estimated by enzymatic method (BQ Kits, San Diego, USA) and INF was measured using Quantiferon TB Gold kit from Cellestis Ltd, USA on Automated ELISA Reader (TECAN, Minilyzer)
Results: RT-PCR was positive in 154 of the 168 cases of tubercular pleural effusions (Sensitivity 91.67%). ADA showed high positivity in 162 of 168 cases (sensitivity 96.42%). INF showed sensitivity of 69.64% (positive in 117 of 168 cases).
The sensitivity of ZN staining in tubercular pleural effusion was 13.10%, Culture for AFB by LJ medium was 31.55% and BACTEC was 57.14%.
Conclusion: Real time PCR provides rapid diagnosis of tubercular pleural effusions. The diagnostic efficiency could be increased by combining RT-PCR with ADA.

P1409

Rapid detection of *Mycobacterium tuberculosis* from clinical specimens using a new target

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Background: Different DNA targets have been introduced for detection of *M. tuberculosis.* Of these targets, *IS*6110 has been used frequently. However, certain strains of *M. tuberculosis*, particularly those identified in Southeast Asia, lack this insertion sequence.
Aims and objectives: Cytochrome P450 CYP141 has not been used as target for detection of *M. tuberculosis*. The aim of this study was to develop a new sensitive and specific PCR method for detection of *M. tuberculosis* directly from respiratory specimens.
Method: The identification of *M. tuberculosis* isolates was confirmed using standard biochemical tests. Primer 3 plus software was used to design primers from CYP141. The expected size of the amplicon was 173 bp. We collected sputa from 247 suspected patients of different cities of Iran.
Results: With the exception of *M. tuberculosis* complex, no amplification was obtained with DNA from other mycobacteria, potentially pathogenic bacteria in the respiratory tract and human cells. These results give evidence that CYP141 can be used as a target for direct detection of *M. tuberculosis* from respiratory specimens. The sensitivity of this target in smear positive- culture positive and smear negative-culture positive samples was 92% and 62.5%, respectively, and the specificity of the PCR was 97.8%.
Discussion: We obtained positive results with a trace amount of template DNA, as little as 1pg. Rv3121 or CYP141 exists in all *M. tuberculosis* isolates used in this study. The high overall specificity (97.8%) and sensitivity (85.7%) of this target in gene amplification illustrates that CYP141 can be a good target for detection of *M. tuberculosis* complex from sputum and possibly other clinical specimens.

P1410

Modification of auramine O fluorescence stain for differential detection of mycobacterium tuberculosis and mycobacteria other than tuberculosis (MOTT)

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Auramine O fluorescent stain was found to be more sensitive than Ziehl Neelsen stain for screening M. tuberculosis directly in sputum specimens, but it lack specificity due to false positivity obtained by mycobacterium other than tuberculosis (MOTT) and weakly acid fast bacteria (e.g: Nocardia species). The aim of this study was to modify the time of decolorization by 0.5% acid alcohol in order to increase the specificity without affecting the sensitivity of the stain. Smears were prepared from 25 bacterial cultures classified into 4 different groups. Group A comprises Mycobacterium tuberculosis complex, group B comprises 15 Mycobacteria other than tuberculosis (MOTT), group C comprises Nocardia farcinica and group D comprises one Gram positive and one Gram negative bacteria. All smears were stained with Auramine O fluorescence stain (IML-Red, Germany), 6 smears from each bacterial isolates were decolorized by 0.5% acid alcohol for (1 m, 2 m, 3 m, 4 m, 5 m, 10 m) to each smear separately, then all smears were counterstained with K permanganate for 1 m. All group (A) bacterial isolates were showed 100% fluorescence after 10 minutes decolorization time while the fluorescence of group (B) isolates were reduced to 73.3% after 5 minutes decolorization time and to 53.3% after 10 minutes. Group (C) showed weak fluorescence after 1 and 2 minutes which completely decolorized after 3 minutes. In conclusion, Mycobacterium tuberculosis complex was resistant to decolorization with 0.5% acid alcohol for 10 minutes while some MOTT were decolorized when using the same time.

P1411

Detection of fluoroquinolone resistance associated mutations in Mycobacterium tuberculosis by use of sequencing and TaqMan probes

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Mutations conferring resistance of MBT to fluoroquinolones occur in two short discrete segments of gyrA and gyrB genes. The nature of the amino acids at positions 88, 90 and 94 in gyrA plays a crucial role in the acquired resistance to quinolones. The aim of investigations is to evaluate the method of real time PCR with dual TaqMan probes for rapid detection of MBT resistance to fluorquinolones.
Methods: Resistance to ofloxacin of MBT was determined by reference technique, gyrA genes were amplified and autosequenced. The real-time PCR with dual TaqMan probes was developed to detect mutations in triplets 90, 94 of the gyrA gene.
Results: The gyrA codons 90, 91, 94 were reported to be the most frequently mutated codons worldwide, the same tendency was registered in Belarus: point mutations were predominately localized at codons 90 and 94 and rarer - 91. Mutations occurred at codon 90 resulted in Ala→Val replacements, at triplet 94 – Asp→Gly or Asp→Asn, at codon 91 - Ser→Pro. The designed dual TaqMan probes for real-time PCR allowed detecting mutations in triplets 90, 94 of gyrA gene. Samples with mutations were characterized by above-threshold florescence on JOE-channel and subthreshold florescence on FAM-channel, while samples without mutations displayed above-threshold florescence on FAM-channel and subthreshold florescence on JOE-channel. The results obtained by real-time PCR with dual TaqMan probes showed high level of coincidence with sequencing data (94%).
Conclusion: The dominant mutations in 90, 94 triplets gyrA can be rapidly and effectively detected by PCR with dual-probe TaqMan probes.

P1412

Resazurin microtitre assay (REMA) plate – A simple, rapid and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis

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Objectives: Comparison of REMA Plate method with the Standard Proportion Method using L-J medium as approved by Revised National Tuberculosis Control Programme (RNTCP) for Drug Sensitivity Testing(DST).
Methods: 26 isolated strains of M. tuberculosis obtained from suspected patients were subjected to DST by 2 methods viz. REMA Plate and Proportion Method for the first line antitubercular drugs INH, RIF, STR and ETM. REMA plate method was performed in 7H9-S medium containing Middlebrook broth and supplements in 96 well microtiter plates. The results of REMA were obtained on days 7, 10 and 14 using resazurin. Any strain having an MIC equal or above the tentative breakpoint concentration was considered resistant.
Results: The sensitivity of REMA for the respective drugs was found to be 94.4%, 93.75%, 93.3% and 66.6%. The specificity of REMA for the respective drugs was found to be 100%, 100%, 45.5% and 35.3%. The cost of REMA Plate method came out to be half of that required for Proportion Method.
Conclusions: REMA can be considered as one of the most rapid and inexpensive method to find out drug resistance to INH and RIF and hence aid in the diagnosis of MDR-TB. ETM and STR, two drugs known to be difficult to test showed a low specificity.

P1413

Evaluation of real time polymerase chain reaction in tubercular mediastinal lymphadenopathy

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Introduction: Mediastinal lymphadenopathy is a common manifestation of tuberculosis. Several other conditions such as Sarcoidosis and lung cancers also cause mediastinal lymphadenopathy. Radiology alone does not provide a diagnosis of TB in such cases. Conventional methods such as culture on LJ medium give results after several weeks while ZN stain for AFB has low sensitivity. We studied the sensitivity of Real Time Polymerase Chain Reaction (RT-PCR) on samples obtained by Trans Bronchial Needle Aspiration (TBNA) through fibreoptic bronchoscopy.
Aims and Objectives: To evaluate the sensitivity of RT-PCR in cases of mediastinal lymphadenopathy due to tuberculosis.
Methods: RT-PCR was performed in 39 consecutive patients of mediastinal lymphadenopathy who underwent fibreoptic bronchoscopy and TBNA of the mediastinal nodes. Final diagnosis of all patients was based on Histopathology, ZN staining and BACTEC culture for AFB.
RT-PCR was performed by detecting amplification reaction for the insert element IS6110 of the *Mycobacterium tuberculosis* complex (Biotub-QT, Biotools Labs, Spain) using a real-time centrifugal amplifcation system (Rotor-Gene 3000, Corbett Research, Australia).
Results: Of the 39 cases of mediastinal lymphadenopathy, 21 were due to tuberculosis. In 11 cases the cause was Sarcoidosis and 7 cases were due to malignancy.
RT-PCR was positive in 19 of the 21 cases of tuberculosis (Sensitivity 90.48%). There was 1 false positive RT-PCR in a case of lung malignancy. The Specificity of RT-PCR in tuberculosis was 94.74%.
Conclusion: Real time PCR is valuable in the diagnosis of mediastinal lymphadenopathy due to tuberculosis with a sensitivity of 90.48% and specificity of 94.74%.

P1414

Xpert MTB/RIF assay for rapid detection of Mycobacterium tuberculosis and rifampicin resistance

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Introduction: Xpert MTB/RIF is a novel molecular diagnostic point of care test for rapid detection of MTB and its susceptibility to rifampicin (RIF).
Objective: This study was performed to evaluate the performance of Xpert MTB/RIF assay in detection of MTB and resistance to rifampicin from sputum specimens taking positive culture for MTB and phenotypic resistance to rifampicin as reference standards.
Materials and methods: 126 consecutive patients of pulmonary tuberculosis presenting to the hospital from December 2010 to January 2012 were enrolled for the study. Their sputum samples were subjected to concentrated ZN microscopy, culture on solid (LJ) and liquid (MGIT) media. All positive cultures were identified as MTB complex using SD TB Ag MPT 64 Rapid immunochromatographic test and indirect drug susceptibility testing performed by MGIT SIRE.
Result: Of the 126 cases included in the study, 83 were smear-positive and 43 were smear-negative. Of patients with culture positive samples, 20 of 126 (15.9%) were found to have multidrug resistance on indirect drug susceptibility testing with MGIT SIRE. With positive culture as the reference standard, MTB/RIF assay when done detected 98.7% of smear positive cases and 72.1% of smear negative cases. The test correctly identified all 20 of rifampicin resistant culture isolates and 105 of 106 rifampicin susceptible isolates for a sensitivity, specificity, positive predictive value and negative predictive value of 100%, 99%, 95.2% and 100% respectively.
Conclusion: Xpert MTB/RIF assay is a reliable technique for rapid detection of Mycobacterium tuberculosis and Rifampicin resistance from pulmonary specimens.

P1415

The prevalence of pyrazinamide resistance in multidrug resistant tuberculosis cases in the Netherlands

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Introduction: Pyrazinamide is very important in the treatment of multidrug resistant tuberculosis (MDR TB). Unfortunately, studies on the prevalence of pyrazinamide resistance in MDR TB cases are scarce and mainly come from non-European countries.
Aims and objectives: Our aim was to determine the prevalence of pyrazinamide resistance in MDR TB cases from the Netherlands.
Methods: We retrospectively analyzed pyrazinamide resistance in all MDR TB cases from the Netherlands found in 2007-2011. Drug susceptibility testing was performed using the Mycobacterial Growth Indicator Tube (MGIT) method. Also, in every resistant isolate the *pncA* gene was sequenced. Resistance was determind using a diagnostic algorithm described recently by our group incorporating both methods (Simons, S.O., *et al*. JCM 2012; 50:428-34).
Results: 61 cases of multidrug resistant tuberculosis were seen in the Netherlands from 2007-2011. Pyrazinamide resistance testing was possible in 59 cases. In 17 MDR TB cases pyrazinamide resistance was observed (29%). 16 out of these 17 cases carried a nonsynonymous mutation in the *pncA* gene.
Conclusions: Our data suggest that among MDR TB cases in the Netherlands pyrazinamide resistance is around 29%. Strategies are required to determine the optimal diagnostic algorithm in this population.

P1416

Comparative analysis of detection of Mycobacterium tuberculosis and rifampin resistance determination through microbiological and molecular genetics methods for pulmonary tuberculosis patients with presence or absence of sputum

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The majority of studies on diagnostics of pulmonary tuberculosis (PTB) used only sputum that appears at later stages of disease and don't use bronchoalveolar lavage (BAL) fluid obtained by bronchoscopes. At the same time these patients are epidemiologically dangerous even at the early stages of disease. The aim of our study was to compare the possibilities of microbiological and molecular genetics methods for Mycobacterium tuberculosis (MTB) detection and rifampin resistance identification (RIF-R) in new cases of PTB through sputum or BAL fluid examination. We conducted a double-blind randomized retrospective investigation. The aetiology of PTB has been proven by receiving the culture growth or histological examination of lung tissue with Ziehl-Neelsen staining. A total of 134 specimens (32 sputum and 102 BAL fluid) from 133 patients gave positive results by Real-Time PCR (kit "AmpliSenceMTB-FL") and by sequencing of rpoB gene (kit "AmpliSenceMTB-Rif-seq"). We received growing of MTB culture in only 23 (74.2%) sputum and 56 (54.9%) BAL fluid compared to the molecular genetics investigations. We found RIF-R in 12 (38.7%) sputum and 37 (36.3%) BAL fluid by all methods. In addition to the culture method we managed to detect RIF-R in 5 sputum and 25 BAL fluid samples (plus one insignificant mutation). Due to the higher sensitivity of molecular genetics methods we not only found out MTB in proven cases of PTB more likely than culture method, but also higher level of RIF-R that did not demonstrate a difference between patients with presence or absence of sputum.

P1417

Assessment of molecular assays for detection of MDR *Mycobacterium tuberculosis* resistant to second-line injectable drugs

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With the emergence of MDR-TB and XDR-TB rapid and accurate second-line drug-susceptibility testing became essential. The [GenoType® MTBDRsl] (Hain Lifescience, Germany) gives the possibility to identify mutations in the *rrs, gyrA* and *embB* genes. The aim of the present study was to assess the sensitivity of this molecular assay for the identification of *M. tuberculosis* resistant to second-line injectable drugs (SLIDs), as, to our knowledge, detection of *rrs* mutations is not sufficient for this purpose. 40 MDR *M. tuberculosis* strains, also resistant to SLIDs, were chosen from our culture collection. 20 SLIDs-susceptible strains were adopted as control. Three DNA targets were investigated: the *rrs* gene, the *eis* promoter region and the *tlyA gene*. The [GenoType®] was used to detect *rrs* mutations and SSCP and sequencing were performed for all three genes. All kanamycin and capreomycin-resistant cultures carried an A1401G substitution in the *rrs* gene and were detected with all the molecular assays, but one of the strains also had an *eis* mutation. 8 kanamycin-resistant strains carried various *eis* mutations, the most common being the C14T substitution, and thus could not be identified with the [GenoType®]. No mutations were found in the *tlyA gene* gene. One of the SLIDs-susceptible strains carried a C12T mutation. Therefore 20% of M. tuberculosis strains resistant to kanamycin could not be detected with the [GenoType®]. Mutations in the *eis* promoter region are found in kanamycin-resistant *M. tuberculosis* strains at high rates and should be included in molecular assays for detection of SLIDs resistance.

P1418

Modern molecular direct tests for rapid identification and drug susceptibility testing of *Mycobacterium tuberculosis*

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Molecular tests are widely used for detection of M. tuberculosis (MTB) in clinical samples in laboratories around the world. At present, there are molecular tests allowing to perform MTB detection and DST to antituberculosis drugs simultaneously.

We performed a comparative analysis of three molecular tests: GeneXpert MTB/RIF (Cepheid, USA), GenoType MTBDRplus (Hain Lifescience, Germany) and TB-Biochip MDR (LLC "BIOCHIP", Russia).
All in all 151 sputum samples were investigated. 32 of them were smear- and culture-positive, and 19 of them were smeare-negative but culture-positive. Coincidence of the results of MTB detection by molecular tests with the culture test in Bactec 960 (Becton Dickinson, USA) were 92,2%, 78,4% and 49,02% for GeneXpert MTB/RIF, TB-Biochip MDR and MTBDRplus respectively. For the smear- and growth-positive sputum samples results coincided in 100%, 96,7%, and 83,3% of cases for GeneXpert MTB/RIF, TB-Biochip MDR and MTBDRplus respectively. For the smear-negative and growth-positive sputum samples results coinsided in 80,9% and 52,4% of cases for GeneXpert MTB/RIF and TB-Biochip MDR respectively.
Results of molecular and bacteriologic DST to rifampicin (RIF) (izoniasid (IHN)) coincided in 100%, 97,4% and 100% of cases for GeneXpert MTB/RIF, "TB-Biochip MDR" and MTBDRplus respectively.
Our data show, that GeneXpert MTB/RIF is the fastest real-time test with the highest sensitivity, but we can get the information about MTB sensitivity only to RIF. As to TB-Biochip MDR and MTBDRplus tests, they are also simple, reliable and allow performing DST to RIF and IHN, but the sensitivity of MTBDRplus is not enough to analyze smear-negative samples.

P1419

Population-based study of fluoroquinolone-resistance in clinical isolates of Mycobacterium tuberculosis in Novosibirsk Oblast, Russian Federation

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Background: Fluroquinolones(FQ) are used for treatment of many bacterial infections frequently. These drugs are being used for treatment of MDR-TB in Novosibirsk Oblast(NO) since 2003.
Aims: To evaluate prevalence of primary drug resistance of M.tuberculosis to FQ in NO. To estimate contribution of common prescription of FQ for treatment of non-TB infections to resistance selection in mycobacterial population.
Methods: Minimal inhibitory concentrations(MIC) of ofloxacin(OFL) were determined for 344 isolates of M.tuberculosis from 344 patients with newly diagnosed TB. This selection consisted from 165 isolates obtained in 2000-2002 and 179 - in 2006-2010. DST was conducted on all isolates and based on results they were divided additionally in to the following groups - 141 fully susceptible to first-line anti-tuberculosis drugs isolates, 83 MDR isolates and 120 resistant other than MDR.
Results: An increase in MIC of OFL(to 16,0-32,0 mcg/ml) was detected in the MDR-group,(7,2%, 95%CI:3,4-14,9) exclusively and only among isolates obtained in 2006-2010 (3,4%, 95%CI:1,5-7,1). FQs have not been prescribed to 6 patients with FQ-resistant MTB (MIC of OFL > 4,0 mcg/ml) within the period prior to establishing of TB diagnosis, 3 from them have had a contact with an MDR-TB patient. The calculated prevalence of primary FQ-resistance of M. tuberculosis in NO was 6,4%, 95%CI:2,9-13,2 (result of the sampling of 94 isolates obtained in 2008-2010).
Conclusions: These findings indicate that emerging FQ-resistance in MBT strains in NO is the result of treatment of patients with MDR-TB rather than from FQ prescriptions to general population for non-TB infections.

P1420

Substantial time reduction in diagnosis of *Mycobacterium tuberculosis* rifampicin and isoniazid resistance by the application of a DNA strip hybridization assay in clinical samples

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The MTBDR*plus* (Hain Lifescience, Nehren, Germany) is a molecular assay detecting mutations involved in *M. tuberculosis* rifampicin (RMP) and isoniazid (INH) resistance, approved for application on culture isolates and smear-positive (AFB+) specimens[1].We evaluated its performance as a routine diagnostic assay directly on clinical specimens.
Methods: Consecutive AFB+ and selected AFB- specimens, from high risk patients for drug resistance, were assayed. The results were compared to conventional drug susceptibility testing (DST). For time reduction estimation we counted the days elapsed before patient's first positive culture was available.
Results: 475 specimens were analyzed. Interpretable MTBDR*plus* results were obtained for 331/400 (82.7%) AFB+, 33/70 (47%) AFB- and 1/5 (20%) microscopically suspicious specimens. We identified: 13 MDR, 28 INH resistant, 2 RMP resistant, and 2 *rpoB* polymorphic strains. Disagreement between DST and MTBDR*plus* mainly involved strains with *inhA* mutations (6/8) conferring resistance to low INH concentrations[2]. The sensitivity, specificity, PPV and NPV values for RMP and INH resistance detection were 100%, 99, 6%, 92, 8%, 100% and 86, 6%, 96, 9%, 79%, 94% respectively. Substantial reduction in drug susceptibility diagnosis was recorded (14.1±6.2 days).
Conclusions: The MTBDR*plus* assay was informative for the great majority of AFB+ cases, highly accurate for screening RMP and INH resistance and its application achieved considerable reduction in diagnostic delay.

[1]Hillemann D. *et al.* J. Clin. Microbiol. 2007, 45: 2635–2640
[2]Hongling G. *et al.* J. Med.Microbiol. 2006, 55: 1527-1531.

P1421

Dual TaqMan probes for the detection of rifampin resistance in *Mycobacterium tuberculosis*

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Mutations (SNP) at codons 526, 531 of rpoB gene of MTB are considered to be biomarkers of resistance to rifampin (RMP). The aim of the study was to develop 96-well plate qPCR platform with dual TaqMan probes for the prediction of RMP resistance in MTB.
Methods: A total number of 122 DNA extracts from MTB cultures were studied. SNPs in 516, 526, 531 codons of rpoB gene were detected by autosequencing and qPCR with dual TaqMan probes (labeled with FAM and JOE for identification of wild and mutated codons correspondingly).
Results: We present a 96-well PCR plate method for identification of SNPs in rpoB gene of MTB. Each 96-well PCR plate is organized as follows: 1) 12 DNA extracts can be investigated collectively; 2) four wells are used for each DNA extract to identify MTB and mutations in 516, 526, 531 codons; 3) one set of controls per 6 DNA extracts is provided including positive, and negative (with and without mutations), and no-template controls for each codon tested. The entire assay includes the following steps: 1) filling wells with DNA-extracts and PCR reaction mixtures using repeater pipette; 2) amplification and data bioanalysis including: a) detection of fluorescence threshold using method of negatives with 20% tolerance; b) allele discrimination analysis based on detection of differences in JOE and FAM fluorescence. The results obtained with this real time PCR design agreed well with DNA sequencing data. Out of 122 DNA extracts (488 reactions) 8 samples were false negative (7%) versus sequencing data. This 96-well PCR plates diagnostic platform resembles ELISA and represents an adequate method for the specific and rapid detection of RMP resistance in MTB cultures.

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Line probe assay (LiPA) based rapid detection of multiple drug resistent (MDR) mycobacterium tuberculosis (MTB)

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Objective: Rapid confirmation of diagnosis of MTB and MDR-MTB in clinical samples by Line probe assays based on Reverse DNA hybridization(RDH)
Methods: RDH is useful for detection of mutations related to drug resistance in Mycobacterium tuberculosis. The sample size of 50 AFB staining positive sputum samples taken was tested. Of these 30% were cases known to be MDR cases as detected from culture tests and 70% were fresh smear positive cases on treatment for TB. The Line probe assays were developed to cover common drug resistant mutations with rifampicin, INH, and aminoglycosides. The genes probed were rpoB, inhA, katG, gyrA, rrs, eis, the MTB complex and NTM. The end point was detected was by avidin biotin labeled nested PCR products from patient sputum samples.
Results: All the 50 smear positive samples were detected as MTB or NTM positive by the LIPA assay. All the known MDR cases showed drug resistance related mutations in the LIPA. About 30% had mutations in the gyrase gene and all were observed in clinically known MDR cases; 46% of mutations were seen in the rpo gene and 56% in the katG region. The efficacy of the line probe when related to culture tests was greater than 90%.
Conclusion: The LIPA assay relates well with both MTB mycobacterial presence and Drug resistance.
References:

[1] Morgan M, Kalantri S, Flores L, Pai M.A:commercial line probe assay for the rapid detection of rifampicin in Mycobacterium tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2005 Jul 28;5:62. Review.