Conclusion: When developing new drugs against Mycobacterium tuberculosis, it is important to note that this bacteria has a solid and chemically resistant cell wall. Therefore, anti-TB agents are specific and do not act on other pathogenic bacteria, and vice versa - the huge variety of available antibiotics does not affect the mycobacteria. For all tested compounds there is no correlation between their antimycobacterial activity and activity against other microorganisms. This indicates that the action of all potent derivatives ((+)-camphor and (-)-fenchones) are specific to the *Mycobacterium tuberculosis*. All of them are stable, non-toxic against human cells and show antimycobacterial activity in the nanomolar range being 60 times more active than ethambutol.These results can be considered an important starting point for design of new effective antitubercular drugs.

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Genotypes of mycobacterium tuberculosis isolated from blood of active tuberculosis patients

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Background: Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. Mycobacteremia is a key event in the pathogenesis of tuberculosis and development of extrapulmonary TB. Genotyping of *M. tuberculosis* isolates is useful for surveying the dynamics of TB infection, identifying new outbreaks, and preventing the disease. We attempted to estimate the prevalence of *M. tuberculosis* bacteremia and its genotypes.

Methods & Materials: Suspected cases of tuberculosis were screened for mycobacteremia by culturing 5 ml of peripheral blood sample in the MB/BacT® culture system (bioMérieux, France). Flashed positive bottles were screened for *M. tuberculosis* using *in-house* multiplex PCR and further screened for drug susceptibility. All isolates were subjected to spoligotyping, 24 loci MIRU-VNTR, $TbD1^{+/1}$ and RD analysis. Genotyping analysis was performed using SIT-VIT web and MIRU-VNTRplus online tools.

Results: Blood culture was performed from 469 patients (306 extrapulmonary, 50 pulmonary and 113 disseminated TB cases). Seventy seven (16.4%) of these blood cultures were positive by MB/BacT®. Of the 77 isolates, 36 (11.7%) were patients had extrapulmonary TB, 34 (30%) disseminated TB, and 7 (14%) pulmonary tuberculosis. Among the culture positives 67 (87%) were identified as *M. tuberculosis* and 10 (13%) as mycobacteria other than tuberculosis using *in-house* multiplex PCR. Spoligotyping identified 65 isolates (97%) belong to 16 previously described Share Types, while the remaining two isolates had unique/un-identified Share Types patterns based upon SIT-VIT web. Among them ST26 (CAS1_DEL) was more predominant (49.2%) followed by ST25 (CAS1_DEL) (9.2%), ST1 (Beijing) (9.2%) and ST19 (EAI2_MANILLA) (7.6%). Largest number of clustering of isolates was detected in Beijing (100%), CAS (97.4%), and EAI (81.8%) by spoligotyping. TbD1 analysis showed that majority of the isolates (83.5%) were modern lineages ($TbD1^{-}$) and 11 (16.4%) isolates belong to ancient linage ($TbD1^{+}$). All the ancient lineage isolates belong to EAI lineage.

Conclusion: CAS lineage was predominantly found among mycobacteremia patients. This pattern is not different from the predominance of mycobacterial isolates genotyped from pulmonary TB cases.

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Diagnostic performance of RT-qPCR method by targeting 85B mRNA in the laboratory diagnosis of Mycobacterium tuberculosis infection: A preliminary study in Turkish patients

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Background: The problems with any PCR method using DNA sequences as targets for amplification include false-positive results, the inability to detect a difference between viable and nonviable organisms and the inability to determine drug susceptibility**.** Instead of DNA detecting assays, using RT-qPCR to assess the expression of the 85B mRNA gene of *M. tuberculosis* indicates the presence of a viable organism. In this study, we used the mRNA coding for 85B antigen complex which is present in all mycobacteria. We aimed to assess the diagnostic performance of RT-qPCR method by comparing with the real-time PCR Cobas TaqMan MTB kit.

Methods & Materials: A total of 54 cases including 32 (17 males, 15 females) patients with confirmed tuberculosis and 22 (13 males, 9 females) individuals without tuberculosis were included as patient and control groups, respectively. The mean ages of 30 males and 24 females were 37,06 ± 8,88 and 36,75 ± 8,96, respectively. Decontamination with N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH) at 2% and Mycobacterial Growth Indicator Tube (MGIT) 960 system was used for the laboratory diagnosis of sputum samples. Decontaminated samples were used for the manual extraction of *M. tuberculosis* DNA and 85B mRNA. Extracted DNA was used for Cobas TaqMan MTB DNA qPCR kit (Roche Diagnostics) in a cobas TaqMan 48 analyzer. For RT-qPCR method, total RNA was extracted from the sputum specimen using standard TRIzol protocol (Invitrogen) and 85B mRNA analyses were performed on LightCycler 480 II system.

Results: A significant difference was detected between patients and control groups by RT-qPCR method ($p<0.001$) and Cobas TaqMan MTB DNA qPCR kit ($p=0.0027$). Sensitivity, specificity, positive and negative predictive values and kappa coefficient were determined as 97%, 95%, 97%, 95%, 0.89% for RT-qPCR method and 91%,

86%, 91%, 86%, 0.96% for Cobas TaqMan MTB DNA qPCR kit, respectively.

Conclusion: Although, our preliminary results indicated that the diagnostic performance of mRNA-based RT-qPCR method is slightly better than the commercial DNA-based diagnostic qPCR assay, we may come to a more definite conclusion after the completion of the study.

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Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of Acinetobacter baumannii outbreak and sporadic isolates

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Background: *Acinetobacter baumannii* is a frequent cause of nosocomial infections, in particular ventilator-associated pneumonia, urinary tract, and bloodstream infections. Patients in high-dependency care are mostly affected, and increasing multidrug resistance worldwide is a cause of growing concern.

Methods & Materials: To compare the two *Acinetobacter baumannii* multi-locus sequence typing (MLST) schemes and to assess their suitability to aid in outbreak analysis we investigated the molecular epidemiology of 99 *Acinetobacter baumannii* isolates representing outbreak-related and sporadic isolates from 24 hospitals in four different countries (Germany, Poland, Sweden, and Turkey). Pulsed-field gel electrophoresis (PFGE) was used as the reference method to determine the epidemiologic relatedness of isolates and compared to MLST using both the Oxford and Pasteur scheme. Rep-PCR was used to define international clonal lineages (IC).

Results: We identified 26 unique outbreak strains and 21 sporadic strains. The majority of outbreaks were associated with carbapenem-resistant *A. baumannii* harbouring oxacillinase OXA-23-like and corresponding to IC 2. Sequence types (STs) obtained from the Oxford scheme correlate well with PFGE patterns, while the STs of the Pasteur scheme are more in accordance with rep-PCR grouping, but neither one is mirroring completely the results of the comparator. On two occasions the Oxford scheme identified two different STs within a single outbreak where PFGE patterns had only one band difference. The CCs of both MLST schemes were able to define clonal clusters that were concordant with the ICs determined by rep-PCR. IC4 corresponds to the previously described CC15 Pasteur (=CC103 Oxford).

Conclusion: It can be concluded that both MLST schemes are valuable tools for population-based studies. In addition, the higher discriminatory power of the Oxford scheme that compares with the resolution obtained with PFGE can often aid in outbreak analysis.

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Non-tuberculous mycobacterial empyema in an immunocompetent child

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Background: Non-tuberculous mycobacterium (NTM) species are free-living organisms that are ubiquitous in the environment. There are about 100 different species of mycobacteria of which few species cause human disease in immunocompromised individuals. The spectrum of disease caused by NTM range from pulmonary disease including cavity, consolidation , bronchiectasis, lymphadenitis, skin, soft tissue and injection site infections to disseminated disease in immunocompromised individuals.

Methods & Materials: We report a 9 years old immunocompetent female patient who presented with *Mycobacterium avium intracellulare* (MAI) empyema. There have been only two reports to our knowledge regarding NTM in immunocompetent children causing empyema both of which were due to mycobacterium chelonae.

Results: A 9 years old girl presented with left sided chest pain for 3 months. There was associated intermittent fever and cough for the last two weeks. Chest X-ray (CXR) revealed massive left sided pleural effusion. Pleural tap drained pus. She underwent thoracotomy and decortication. HIV Elisa was negative. TB MGIT culture at the end of 3 weeks grew non-tuberculous slow growing mycobacteria. Line probe assays revealed MAI. A thorough immune workup showed normal serum levels of IgG and IgM and lymphocyte subset assays. Child was started on clarithromycin, isoniazid, rifampicin and ethambutol. A repeat chest X-ray done after two months showed complete resolution of pleural fluid. Treatment is continued and child is on regular follow up.

Conclusion: MAI can lead to tuberculous empyema even in immunocompetent children.

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Ocular tuberculosis masquerading as retinoblastoma in a young boy

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Background: Ocular involvement in childhood tuberculosis is rare. We report a case of intra ocular tuberculosis in a young boy who presented with ocular discomfort and blurring of vision and was diagnosed as retinoblastoma preoperatively. Child underwent