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Abstracts' Service

Evaluation of Manual Mycobacterium Growth Indicator Tube for Isolation and Susceptibility Testing of *Mycobacterium Tuberculosis* for Implementation in Low and Medium Volume Laboratories

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Background. Manual *Mycobacterium* growth indicator tube (MGIT) was evaluated for isolation and drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) for its implementation in laboratories with low and medium volume.

Methods. 1018 consecutive clinical specimens were processed using manual MGIT and conventional Lowenstein–Jensen (LJ) culture. Results obtained for culture positivity were analyzed taking combined reference of positivity by either solid or liquid culture. All positive cultures were identified and DST to first line drugs was performed by manual MGIT and 1% proportional method on LJ media. Performance of manual MGIT for DST was compared to conventional DST on LJ media.

Results. Of the total 220 culture positive samples 93.9% were isolated in MGIT while 75.7% in LJ taking combined reference of positivity by either solid or liquid culture. Turn around time for isolation of MTB was significantly less for MGIT as compared to LJ. There was good agreement between manual MGIT and 1% proportional method on LJ media for DST to first line drugs. Turnaround time from inoculation to DST results for smear positive and smear negative cases using manual MGIT was 20.2 and 30.1 days respectively. The total cost for isolation, identification and DST in manual MGIT for smear positive and smear negative cases was INR 2350 and INR 2700 respectively.

Conclusion. It is feasible to implement manual MGIT in low to medium volume laboratory that already has experience with culture provided adequate biosafety measures and appropriate training of laboratory staff are taken care of.

Pharmacokinetics of Colistin in Patients with Multidrug-resistant Gram-negative Infections: A Pilot Study

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Background & objectives. There is little information concerning intravenously (i.v.) administered colistin in patients with multidrug-resistant (MDR) Gram-negative infections. Thus, this pilot prospective study was undertaken to characterize efficacy and pharmacokinetics of colistin in patients with MDR Gram-negative infections.

Methods. Nine patients with age >12 yr and MDR Gram-negative infections were included, of whom six were given colistin at the doses of 2 MU, while three patients were given 1 MU i.v. dose every 8 h. Blood samples were collected at different time intervals. Determination of colistin concentration was done by a ultra-high-performance liquid chromatography/mass spectrometry/selected reaction monitoring assay.

Results. The area under the plasma concentration-versus-time curve over eight hours (AUC0-8) for colistin after the 1st dose ranged from 3.3 to 16.4 mg×h/l (median, 4.59). After the 5th dose, AUC0-8 for colistin ranged from 4.4 to 15.8 mg×h/l (median, 6.0). With minimal inhibitory concentration (MIC) value of 0.125 mg/l, AUC0-8/MIC ranged from 26.7 to 131.4 (median, 36.7) and 35.5 to 126.0 (median, 48.0) after the 1st and the 5th doses of 2 MU every 8 h, respectively.

Interpretation & conclusions. As there is a paucity of information on AUC/MIC for colistin, it may not be possible to conclude whether AUC/MIC values in our patients were adequate. There is a microbiological clearance of organism, which goes in favour of the dosing schedule being adequate. Further studies need to be done to understand the pharmacokinetics of colistin in patients with infections.

Prevalence and the Risk Factors of Gastro-esophageal Reflux Disease in Medical Students

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Background. Gastroesophageal reflux disease (GERD) is a commonly prevalent gastrointestinal disorder in adults. Very few studies on magnitude of GERD in student community have been done and there is none so far from India. Rigorous MBBS curriculum makes medical students prone for reflux symptoms. Hence, this study was conducted to determine the prevalence of GERD in medical students and the potential risk factors associated with it.

Methods. This was a cross sectional observational study conducted on medical students in a premier medical college of India. All participants were interviewed for GERD symptoms using the validated questionnaire on frequency scale for the symptoms of GERD. Additional 11 questions include enquiries on medical history and lifestyle factors.

Results. Of the 600 students, 150 (25%) had GERD symptoms. Of these, 88 (58.6%) had mild, 58 (38.6%) moderate, and 4 (2.7%) severe reflux symptoms. Fifty eight (38.6%) of students with GERD had associated dyspepsia. On univariate analysis higher BMI, final years of MBBS course, use of NSAID or alcohol, inadequate sleep, sleeping within one hour of taking dinner, missing breakfast regularly and quick eating were significantly associated with GERD ($p < 0.05$).

Conclusions. Prevalence of symptoms of GERD in medical students is 25%, majority had mild symptoms. Associated dyspeptic symptoms were present in 38.6%. Factors predisposing to GERD in them are higher BMI, final years of MBBS course, use of NSAID, inadequate sleep, sleeping within one hour of taking dinner, missing breakfast on regular basis and quick eating.

Association of Furanone C-30 with Biofilm Formation & Antibiotic Resistance in *Pseudomonas aeruginosa*

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Background & objectives. *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause nosocomial bloodstream infections in humans. This study was aimed to explore the association of furanone C-30 with biofilm formation, quorum sensing (QS) system and antibiotic resistance in *P. aeruginosa*.

Methods. An in vitro model of *P. aeruginosa* bacterial biofilm was established using the standard *P. aeruginosa* strain (PAO-1). After treatment with 2.5 and 5 μg/ml of furanone C-30, the change of biofilm morphology of PAO-1 was observed, and the expression levels of QS-regulated virulence genes (lasB, rhlA and phzA2), QS receptor genes (lasR, rhlR and pqsR) as well as QS signal molecule synthase genes (lasI, rhlI, pqsE and pqsH) were determined. Besides, the AmpC expression was quantified in planktonic and mature biofilm induced by antibiotics.

Results. Furanone C-30 treatment significantly inhibited biofilm formation in a dose-dependent manner. With the increase of furanone C-30 concentration, the expression levels of lasB, rhlA, phzA2, pqsR, lasI, rhlI pqsE and pqsH significantly decreased in mature biofilm bacteria while the expression levels of lasR and rhlR markedly increased. The AmpC expression was significantly decreased in both planktonic and biofilm bacteria induced by imipenem and ceftazidime.

Interpretation & conclusions. Furanone C-30 may inhibit biofilm formation and antibiotic resistance in *P. aeruginosa* through regulating QS genes. The inhibitory effect of furanone C-30 on las system appeared to be stronger than that on rhl system. Further studies need to be done with different strains of *P. aeruginosa* to confirm our findings.

AdeR-AdeS Mutations & Overexpression of the AdeABC Efflux System in Ciprofloxacin-Resistant *Acinetobacter baumannii* Clinical Isolates

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Background & objectives. Overexpression of efflux pumps is a cause of acquired resistance to fluoroquinolones in *Acinetobacter baumannii*. The present study was done to investigate the presence and overexpression of AdeABC efflux system and to analyze the sequences of AdeR-AdeS regulatory system in ciprofloxacin-resistant *A. baumannii* isolates.

Methods. Susceptibility of 50 clinical *A. baumannii* isolates to ciprofloxacin, imipenem, ceftazidime, cefepime and gentamicin antimicrobials was evaluated by agar dilution method. Isolates were screened for the evidence of active efflux pump. Isolates were also examined for adeR-adeS and adeB efflux genes by polymerase chain reaction (PCR). The adeR and adeS regulatory genes were sequenced to detect amino acid substitutions. Expression of adeB was evaluated by quantitative reverse-transcriptase PCR.

Results. There were high rates of resistance to ciprofloxacin (88%), ceftazidime (88%), cefepime (74%) and imipenem (72%) and less resistance rate to gentamicin (64%). Phenotypic assay showed involvement of active efflux in decreased susceptibility to ciprofloxacin among 16 isolates. The 12.27-fold increase and 4.25-fold increase were found in adeB expression in ciprofloxacin-full-resistant and ciprofloxacin-intermediate-resistant isolates, respectively. Several effective mutations, including A91V, A136V, L192R, A94V, G103D and G186V, were detected in some domains of AdeR-AdeS regulators in the overexpressed ciprofloxacin-resistant isolates.

Interpretation & conclusions. The results of this study indicated that overexpression of the AdeABC efflux pump was important to reduce susceptibility to ciprofloxacin and cefepime in *A. baumannii* that, in turn, could be triggered by alterations in the AdeR-AdeS two-component system. However, gene expression alone does not seem adequate to explain multidrug resistance phenomenon. These results could help plan improved active efflux pump inhibitors.