Clinical microbiology

Cod: 0414

ASSESSMENT OF THE ANTIBACTERIAL EFFECTS OF MORINGA PEREGRINA EXTRACTS

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BACKGROUND: The antibacterial effect of Moringa peregrina (leaves, roots and seeds) ethanolic extracts were investigated.

METHODS: The effect of plant extracts were tested against three bacterial species: Escherichia coli (E. coli ATCC 25922), Staphylococcus aureus (S. aureus ATCC 43300) and Klebsiella pneumoniae (K. pneumoniae ATCC 13883).

RESULTS: Moringa peregrina ethanolic extracts showed significant antibacterial effect on the three tested bacterial strains using the disc diffusion methods. The inhibition zones caused by leaf ethanolic extracts were 14-30, 8-19 and 9-22 mm in diameter against E. coli, K. pneumonia, and S. aureus respectively. Root ethanolic extracts showed inhibition zones as 18-42, 44-59 and 34-45 mm in diameter against E. coli, K. pneumonia, and S. aureus respectively. Seed extract caused inhibitory zones of 16-38, 6-32 and 6-18mm in diameter against E. coli, K. pneumonia, and S. aureus respectively. The results showed that the zones of inhibition for the three bacterial species increased in a dose dependant manner and that the Moringa peregrina root ethanolic extract exhibited more potent inhibition. The other test done to assess the antibacterial effect of Moringa peregrina ethanolic extracts was the minimum inhibitory concentrations (MIC). Such test was conducted on the same three bacterial species, where the MIC for the Moringa peregrina leaf extract against E. coli, K. pneumoniae and S. aureus were 12.0 mg/ml, 15.0 mg/ml and 18mg/ml respectively. The MIC for seed ethanolic extract were 13.0 mg/ml, and 7.0 mg/ml against E. coli, K. pneumoniae and S. aureus respectively. Also, the MIC for root ethanolic extract of Moringa peregrina against E. coli, K. pneumoniae and S. aureus were, 9.0 mg/ml and 3 mg /ml, 2 mg/ml and 5 mg/ml respectively.

CONCLUSIONS: Such low MIC values especially for the root extract represent strong potential for Moringa peregrina as an antibacterial agent.

Clinical microbiology

Cod: 0415

MOLECULAR CHARACTERIZATION OF ESBLS PRODUCED BY KLEBSIELLA PNEUMONIA STRAINS IN TUNISIA

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BACKGROUND: ESBLs are most commonly detected in Klebsiella pneumoniae, cause of significant community-acquired and hospital-acquired infections. In Tunisia, ESBL-producing K.pneumoniae (ESBL KP) has been described as one of the most important pathogens responsible of serious endemic and epidemic nosocomial infections, especially in neonatal units. In this study, we investigated the molecular epidemiology and characteristics of ESBLs producing Klebsiella pneumonia in Tunisia.

METHODS: A total of 118 clinical isolates of ESBL positive K.pneumonia were collected from different wards between 2009 and 2011. Antimicrobial susceptibilities of the strains were determined according to the CA-SFM. Multiplex PCR was performed to identify blaTEM, blaSHV and blaCTX-M genes. For CTX-M positive strains, PCR was carried out to identify CTX-M 1 group. SHV, TEM and CTX-M were sequenced.

RESULTS: From the 118 strains, 105 (89%) carried blaSHV, while 95 (81.35%) and 67 (56.77%) have blaCTX-M and blaTEM respectively. Co-production of these enzymes was found in 53 (44.91%) strains. All CTX-M positive belonged to CTX-M-1 group. Sequencing results revealed that all CTX-M enzyme types were CTX-M15. Among 105 strains SHV positive, 2 strains carried SHV-11 and SHV-28 and the others SHV-1. Of the 67 strains TEM positive, 2 were TEM-158 and TEM-53, and others TEM-1.

CONCLUSIONS: Our results suggest that CTX-M-15 is emerging among ESBLs-producing Klebsiella pneumonia. To our knowledge, this is the first report of TEM-158 and TEM-53 in Tunisia.

Clinical microbiology

Cod: 0417

STUDY ON MULTIDRUG RESISTANT, EXTENDED SPECTRUM BETA-LACTAMASES AND AMPC BETA LACTAMASES PRODUCING UROPATHOGENES AMONG CHILDREN

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BACKGROUND: Extended spectrum beta-lactamases (ESBL) and AmpC beta-lactamases (AmpC) are the most significant enzymes involved in conferring resistance to Beta-lactam antibiotics in Gram negative bacteria. This study was aimed to find out the prevalence of multidrug resistant (MDR), ESBL and AmpC beta-lactamases producing isolates among children with UTI in Nepal.

METHODS: 820 urine specimens were obtained from clinically suspected UTI children (age< 12 years, female to male ratio 2.1:1. Samples were midstream urine, 25supra pubic aspiration and 4 from catheter. Culture, organism identification and antibiotic susceptibility test were done by following the protocol of American Society for Microbiology (ASM).Isolates resistant to two or more antibiotics were defined as MDR, among them resistant to third generation cephalosporins were further tested for ESBL and AmpC phenotypes.

RESULTS: Among 820 urine samples, 23.51% (201/820) had significant bacterial growth with 184 (91.54%) non-repeat gram-negative isolates in which most were E. coli (58.15%) followed by klebsiella species (15.2%).The prevalence of MDR, ESBL and AmpC were 115 (62.5%), 43 (23.36%) and 15 (8.15%) respectively. Maximum incidence of ESBL producer was E.coli (39.5%) followed by kleibiella (16.2%) and pseudomonas species (13.9%). ESBL producers and non producers MDR isolates were highly resistant to amoxycillin-clavunic acid, aztrenome, cefepime and ceftazidime-clavunic acid. Imepenum and pipracilin was most effective drug among ESBL producers and non producers.

CONCLUSIONS: Result shows alarming high percentage (62.5%) of MDR pathogens in childhood UTI. High prevalence ESBL and AmpC enzymes has significant role in MDR. Routine screening method for ESBL and AmpC are strongly recommended for phenotype screening.

Clinical microbiology

Cod: 0418

LABORATORY DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFECTION

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BACKGROUND: Clostridium difficile infection (CDI) is a major infectious concern, accounting for substantial morbidity. Advances in microbiological and molecular techniques have resulted in an increasing number of testing options for CDI. The purpose of the present study was to report our experience in the diagnosis of CDI from patients hospitalized with diarrhoea, to investigate pathogenicity and virulence of positive strains and to highlight the clinical features of positive cases.

METHODS: Fecal samples from seven hundred forty-four patients were tested at a 883- bed University Hospital in Greece over a period of three years. A commercially available combined Glutamate Dehydrogenase Antigen (GDH) and toxin A/B membrane EIA assay (CDiff Quick Chek Complete, QCC, Techlab, Blacksburg) were performed. Twenty negative [GDH-/ toxin A/B(-)] and all positive samples by QCC test [GDH+/ toxin A/B(+) or GDH+/ toxin A/B(-)] were then tested by PCR (GenoType CDiff, Hain LifeScience, Germany) to perform the molecular genetic identification of C. difficile strains.

RESULTS: Thirty stool specimens from consecutive patients (4%) were tested positive with GDH and PCR and six more were only with GDH. The PCR assay showed complete concordance with GDH+/toxin A/B+ (n=30) and GDH-/ toxinA/B- results (n=20). Of the GDH+/ toxinA/B- specimens 25% were negative by PCR. Both A and B toxins were detected in 80% of all PCR-positive samples and binary toxin genes cdtA and cdtB were also detected in 30% of them. The GenoType CDiff assay was able to identify a strain of ribotype 078 with detectable binary toxin but no one strain were found to be hypervirulent (ribotype 027). Males and children were 56.6% and 6.6% respectively and 89.3% of adults aged more than 65 years. Twenty-five cases were hospital onset and the majority of them derived from Medical wards (64%). Twenty-eight cases (93.3%) had received antibiotics in the preceding month and 73.3% had underlying disease.

CONCLUSIONS: The combined CDiff Quick Chek Complete test can be used as an initial screening test followed by confirmation with PCR assay of GDH positive and toxin-negative samples. Additionally, PCR-GenoType CDiff allows the differentiation between non-pathogenic, virulent and hypervirulent C. difficile strains.

Clinical microbiology

Cod: 0419

EXTENDED SPECTRUM β-LACTAMASE (ESBL) AND METALLO β –LACTAMASE (MBL) PRODUCING GRAM NEGATIVE BACTERIAL ISOLATES CAUSING URINARY TRACT INFECTION IN TERTIARY CARE HOSPITAL

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BACKGROUND: Urinary Tract Infection (UTI) is one of the most common diseases encountered worldwide and is a major public health problem in terms of morbidity and financial costs. During the last two decades bacterial drug resistance mediated by extra chromosomal genetic elements (plasmids), which simultaneously carry gene for resistance to number of antibiotics has made the position miserable, specifically in gram negative bacteria. The updated knowledge and situation of the prevailing bacterial uropathogens that are multi drug resistant (MDR) is of prime importance for the proper use of antimicrobial drugs and the policy making to combat multidrug resistance in UTIs.

METHODS: A prospective cross-sectional study was carried out from Aug 2012 to Jan 2013 in Department of Microbiology at Tribhuvan University Teaching Hospital (TUTH). A total of 1,063 urinary tract samples (Mid stream urine, catheter, and suprapubic aspirate) from patients visiting TUTH were included in this study. Samples were processed for routine microscopy and culture. Then the organisms were identified by standard microbiological methods. Antibiotic susceptibility testing was done by Kirby-Bauer Disk Diffusion method according to CLSI guidelines. ESBL producing organisms were detected by Combination Disk method and MBL producing organisms were detected by Ethylene diamine tetra acetic acid(EDTA)-Imipenem combined disk assay.

RESULTS: Of the total 1,063 samples processed, 26.0% showed significant bacteriuria. Twelve different genera of bacteria were isolated among which E.coli (57.6%) was the most common isolate followed by Staphylococcus aureus (14.1%), Klebsiella spp (6.2%), Enterococcus faecalis (5.4%), Staphylococcus epidermidis (3.9%), Pseudomonas aeruginosa (3.3%), Acinetobacter spp (2.5%), Enterobacter spp (2.2%), Burkholderia cepacia Complex (1.8%), Staphylococcus saprophyticus (1.4%), Citrobacter spp (1.1%) and others (<1%). Among the gram negative isolates (210), majority (61.0%) were found MDR. Most of the ESBL and MBL producing isolates were detected in E.coli and Klebsiella spp respectively.

CONCLUSIONS: There is an increasing resistance to many antibiotics in the both community and hospital settings. Now-a- days, ESBL and MBL producing uropathogens are emerging.

Clinical microbiology

Cod: 0420

THE TIME DETECTION OF MRSA IN 3 DIFFERENT PRE-INCUBATION PERIOD IN THE BLOOD CULTURE BOTTLES

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BACKGROUND: It was indicated that If the blood culture time results prolonged to inform the clinicians, the mordality and morbitidy rate should be effected. The MRSA infections could be identified immediately especially for the hospitalized patients because of the clinician could be change the treatment and reduce the patient's healing. It was important to identified the blood culture results early to reduce the mortality and morbidity rates. The aim of this study was to determine the early signal of the pre-incubation (4 ^{o}C, 25 ^{o}C and 37 ^{o}C) blood culture bottles in 24 hours incubation.

METHODS: In this study, we used 19 MRSA strains which isolated in Microbiology Laboratory of the Near East University Hospital. All the strains were identified by using Phoenix 100 (Becton Dickinson, USA). We determine three pre-incubation degree; I. group: 4^{o}C, II. group: 25 ^{o}C and III. group: 37 ^{o}C. All bottles were pre-incubated in 24 hours before the contamination with MRSA strains. All groups were included 19 MRSA contaminated blood culture bottles and 1 non-contaminated blood culture bottle for the negative control. MRSA strains were suspansion McF 0.5 in brain heart infusion broth. We added 10 mL of 0.5 McF MRSA suspansion in each blood culture bottles. All contaminated and negative control blood culture bottles were placed in the BACTEC 3D ALERT (Becton Dickinson, USA) system.

RESULTS: According to the 3 different pre-incubation groups, the early signal were seen in the 37 ^{o}C blood culture bottles (ANOVA, Tukey's Multiple Comparison Test, F= 8.927, dF= 6,084, **; P<0.05).

CONCLUSIONS: It was reported that MRSA infections are still significance about the mortality and morbidity. We indicate that blood culture bottles which pre-incubated in 37 ^{o}C in 24 hours could be acquire early time to identified the MRSA infections. Unless, the firm declare that the blood culture bottles should be save in room temperature. So that, in the further study, we should analizy the media into the blood culture bottles to understood if the media affected in pre-incubation degrees.

Clinical microbiology

Cod: 0422

THE DETECTION OF SYNERGY OF COLISTIN COMBINATIONS AGAINST GRAM NEGATIVE BACTERIAL PATHOGENS USING AN E TEST METHODOLOGY

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BACKGROUND: Infections due to multi-drug resistant (MDR) pathogens have become a therapeutic challenge. The purpose of this study was to detect the possible synergistic activity of the unconventional combination of colistin (COL) with daptomycin (DAPT) against Gram negative type strains and MDR clinical isolates. We studied also the potential synergy of COL plus meropenem (MEM) versus MDR Klepsiella pneumoniae strains.

METHODS: We tested 31 clinical isolates, resistant to carbapenems and other antimicrobial classes. Ten Pseudomonas aeruginosa, 11 Acinetobacter baumannii and 10 K. pneumoniae strains as well as respective antibiotic susceptible type strains, were selected to evaluate the COL/DAPT synergy. The K. pneumoniae clinical isolates were also used to study the synergistic activity between COL and MEM. Identification and susceptibility testing were performed using the Vitek 2 system (bioMerieux, France). MICs of COL, MEM and DAPT were confirmed by E-test (AB Biodisk), according to CLSI and EUCAST breakpoints. Synergy testing was performed by an E-test MIC/MIC ratio method. The fractional inhibitory concentration index was calculated: synergy <= 0.5, indifference >0.5-4 and antagonism >4.

RESULTS: All isolates had a DAPT MIC >256 μg/mL. The MIC range of COL was 0.047-128, 0.5-1 and 0.047-64 μg/mL for A. baumannii, P. aeruginosa and K. pneumoniae isolates, respectively. All K. pneumoniae clinical strains were MEM resistant (MICs 8->32 μg/mL). Regarding the COL/DAPT combination, 3 (23%) A. baumannii and 1 (9%) K. pneumoniae strains showed synergy, while 10 (77%) and 10 (91%) respectively, indiffererence. All A. baumannii isolates demonstrated a reduction in the MIC of COL, while in 5 strains the MIC of DAPT was decreased down to the level of 16 μg/mL. In none of P. aeruginosa strains detected synergy. The COL/MEM combination showed synergy in all K. pneumoniae isolates and was also able to remarkably reduce the MIC of MEM to less than the susceptibility breakpoint for 2 strains.

CONCLUSIONS: Regimens containing DAPT may confer therapeutic benefit for treating infections due to MDR A. baumannii strains. More extensive work is needed to confirm this impression as well as to investigate whether the in vitro synergy translates into in vivo effectiveness.

Clinical microbiology

Cod: 0424

ENUMERATION AND SURVIVAL OF LACTIC ACID BACTERIA IN PROBIOTIC YOGURTS

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BACKGROUND: A number of health benefits have been claimed for probiotic bacteria such as Lactobacillus acidophilus, streptococcus thermophilus and bifidobacteria. Because of the potential health benefits, these organisms are increasingly incorporated into dairy foods like yoghurts. However, studies have shown low viability of probiotics in the market. Thus survival and presence of lactic acid bacteria, Streptococcus thermophilus and bifidobacteria in yoghurt was investigated

METHODS: Bacteria were isolated from three different products samples, Morrisons, Actimel and Waitrose. All products were analysed before the end of their shelf-life (before the expiry date). Bacteria were isolated with MRS, M17 and RCA agar media, MRS Agar and RCA Agar was incubated anaerobically for 48h and 72hr and M17 was incubated aerobically for 24h. Isolates from M17, RCA, and MRSA were all tested for gram stain reaction, colony appearance, cell morphology, catalase test and carbohydrate fermentation pattern using the API-50 CHL identification system.

RESULTS: The results show that all the three products contained L. acidophilus, S. thermophilus and bifidobacteris. All the organisms test positive to gram staining and negative to oxidase test.

CONCLUSIONS: The organisms tested are present and survive in the products tested
Key words: Lactic Acid Bacteria, Bifidobacteria, Lactobacillus acidophilus, and streptococcus thermophilus, Probiotics, selective enumeration, survival
Abbreviation key: LAB- Lactic acid bacteria, MRS- deMann Rogosa and Sharpe agar, RCA- reinforced clostridial agar.

Clinical microbiology

Cod: 0425

NOVEL LABORATORY DIAGNOSIS FOR NEISERRIA GONOCOCCUS IN MALE PATIENTS SUSPECTED OF SUFFERING FROM GONORRHEA

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BACKGROUND: Failure to isolate Neiserria gonococcus from swab specimens sent to microbiology laboratory, from patients suspected of suffering from gonorrhoea is a great challenge to Medical Scientist, especially in developing countries with little knowledge of modern technology for quick diagnosis for life threatening infections. Moreover, it takes a minimum of 72 hours to make a provisional diagnosis form swab specimens sent to the laboratory. This delay can be worrisome to patients suffering from acute gonorrhoea. The purpose of this study therefore, was to determine the possibility of reducing the time spent in diagnosis of gonorrhoea in patients with purulent urethral discharge, suspected of suffering from gonorrhoea.

METHODS: One hundred and fifty male adults with purulent urethral discharge suspected of suffering from gonorrhoea, who, were attending out patients depart of University of Benin Teaching Hospital Benin City Nigeria, were randomly recruited for the study. Their consent was obtained verbally and were assured of strict confidentiality before study commenced. Also ethics committee approval from University of Benin ethics committee was received. Pus from urethral of the patients was collected using sterile cotton wool swabs and inoculated directly onto pre-warmed chocolate agar plates kept at 37^{o}C, antibiotic discs were added and plates were incubated immediately at 37^{o}C in microaerophylic atmosphere for 24hours. Slides were also made from the swabs and stained by Grams method immediately.

RESULTS: all the specimens yielded a pure heavy growth of cream coloured colonies , identified as Neiserria gonococcus, using oxidase reagent Gram stain revealed intra and extra cellular gram negative diplococcic morphologically resembling Neiserria gonococcus. The antibiotic susceptibility pattern was recorded and the results were sent to their Physician for immediate therapy.

CONCLUSIONS: This study presents, a novel method for the diagnosis of Neiserria gonococcus. The routine method of diagnosis that normally takes 72hours for a provisional diagnosis was reduced to 24hours.
KEY Words; Neiserria, gonococcus Novel Laboratory diagnosis Male patients, Urethral Discharge.

Clinical microbiology

Cod: 0426

DEVELOPMENT OF SINGLE-STEP REAL-TIME PCR-BASED MELTING CURVE ANALYSIS FOR HBV GENOTYPING AND EVALUATION OF ITS APPLICATION IN FUJIAN CHINA

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BACKGROUND: HBV genotyping is an essential part of long-term disease prognosis prediction and personalized medicine. The aims of this study were to establish a novel method for HBV genotyping by real-time PCR-based melting curve in a single-tube reaction and analyze the effect of genotypes on the prognosis of HBV-infected patients in Fujian, China.

METHODS: The primers were designed based on the alignment result of published sequences of genotypes B and C. HBV can be genotyped by different Tm values of PCR products. The sensitivity, specificity and reproducibility of the assay were evaluated. The association of HBV genotypes with the severity of liver disease, HBeAg status and HBV DNA load were also explored.

RESULTS: The Tm values was 80.79 ± 0.35°Cfor HBV genotype B and 85.75 ± 0.33°C for genotype C. The CV (coefficient of variation) values for Tm in high, medium and low plasmid DNA were 0.15%, 0.09%, 0.16%, respectively. Compared to a commercial kit, the concordance rate was 100%, 100% and 81.25% for genotype B, genotype C and genotype B/C mixed respectively. The overall concordance between the two assays was 94.12% (48/51, Kappa =0.909, P<0.05). Genotype B, C and B/C mixed accounted for 54.91% (190/346), 23.70% (82/346) and 21.39% (74/346), respectively among the 346 subject population. The HBeAg-positive rate and HBV DNA concentration of genotype C were significantly higher than genotype B.

CONCLUSIONS: The established PCR-based melting curve assay was a promising tool for HBV genotyping in the areas prevalent with HBV genotype B and C. HBV genotype C tended to conduct more severe liver disease than genotype B.
Keywords: Hepatitis B virus; Genotype; Melting curve analysis; Liver fibrosis; HBeAg.

Clinical microbiology

Cod: 0427

LABORATORY INVESTIGATION OF BRUCELLOSIS IN RURAL AREAS IN NORTHERN GREECE

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BACKGROUND: Brucellosis is a zoonosis, caused by several species of Brucella bacteria, that have their natural reservoir in domestic animals. Brucella melitensis is the most pathogenic and invasive one. Brucellosis is a major problem in the Mediterranean region, in particularly in the agricultural and pastoral populations. Greece is one of the Mediterranean countries where brucellosis is endemic and constitutes a serious public health problem as well as an economic one in several rural areas. The present study aimed to evaluate the seroprevalence and examine the risk factors of brucellosis in α rural area in northern Greece.

METHODS: The participants in the study were 108 (80 men/28 women). The following information was obtained from each person: sex, age, occupation, contact with animals, consumption of unpasteurized products, loss of weight, history of fever, weakness, myalgia, joint pain, fatigue, hepatomegaly, splenomegaly and lymphadenopathy. All sera were examined using the standard serum agglutination test (SAT) according to the manufacturer's instructions. Two criteria were basic to establish the disease. The first one was clinical symptoms and the second was a titer of SAT at least 1:160.

RESULTS: Of the 108 subjects examined, 14 (12,9%) were seropositive. The age of the seropositive people was (mean age +/- standard deviation) 44,24 +/- 18,267. The overall prevalence differed between males (11,1%) and females (1,8%). No seropositivity was observed in the >70 year old age group. Farmers and stock breeders had higher prevalence of antibodies compared with other professions (12,1% and 16,6%, respectively). One chronic case was detected (SAT – Coombs positive).

CONCLUSIONS: The detection of brucellosis in animals is essential for the prevention of the disease. In addition efficient preventive measures should be established in order to eliminate the disease.

Clinical microbiology

Cod: 0428

ISOLATION AND IDENTIFICATION OF PATHOGENS IN CLINICAL BACTERIOLOGY LABORATORY: AN EXTERNAL QUALITY ASSESSMENT PERSPECTIVE

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BACKGROUND: External quality assessment (EQA) consists of analysis of identical specimens by the participating laboratories and expert evaluation of the results. While the specimens simulate real life cases, results from EQA provide an invaluable tool in assessing laboratory performance. This study presents the design of a clinical bacteriology EQA scheme organized by Labquality and reviews the results from 2008-2013.

METHODS: General Bacteriology 1 scheme includes isolation of aerobes and anaerobes from lyophilized mixtures of pathogens and normal flora as well as antimicrobial susceptibility testing. The rounds consist of 4 specimens simulating different infection focuses. Findings range from bacteria to yeasts and molds, one sample containing one or several pathogens. On average 70-90 laboratories from 10 countries participate to the EQA rounds.

RESULTS: During 2008-2013 more than 80 different microbes were included in the 96 specimens assessed. The findings included common microbes, e.g., Escherichia coli, Enterobacter aerogenes and Enterococcus faecalis but also rarer species, e.g., Capnocytophaga canimorsus, Actinomyces odontolyticys and Actinomadura madurae . In general, anaerobes and less common aerobes caused most difficulty. Common findings such as E. coli and E. faecalis were identified in about 90 % of the laboratories whereas less than 20 % identified rarer pathogens, e.g., A. odontolyticus. The overall performance of antimicrobial susceptibility testing was good. However, both false resistance and sensitivity was often reported by some of the laboratories probably telling of inadequate adherence to the methodological standards.

CONCLUSIONS: EQA provides clinical laboratories an important tool in ensuring competence and in gaining experience in less commonly encountered clinically relevant cases. By repeating similar specimens and microbes in short intervals the participants can monitor possible trends in performance and make the required changes. While less than 20 % of the laboratories are capable of identifying certain types of pathogens, significant opportunities for learning remain. Participation in comprehensive and educational EQA is recommended for all clinical laboratories.

Clinical microbiology

Cod: 0429

EMERGENCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAEAT THEUNIVERSITY HOSPITAL CENTER OF BLIDA

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BACKGROUND: Carbapenemantibiotics are used as the last line of treatment for infections caused by Enterobacteriaceae multidrug-resistant. However, the emergence of bacteria resistant to this important group of drug is jeopardizing the use of carbapenems. Resistance to carbapenems is due to production of enzymes Carbapenemases. Another important cause among carbapenem-resistant Enterobacteriaceae (CRE) is over production of ESBL (extended spectrum β-lactamases) or Amp C enzyme in organism with porin loss. Phenotypic detection of CRE is important for proper infection control and appropriate patient management. The present study detects CRE by simple phenotypic method.

METHODS: The study was a prospective study carried out at the Laboratory of Microbiology, at the university hospital center of Blida, from January 2012 to January 2014. The susceptibility to antibiotics was carried out according to recommendations of Clinical Laboratory Standard Institute by: disk diffusion method, search of ESBL and evaluation of the minimal inhibitory concentration (MIC). Resistance of Enterobacteriaceae to carbapenems was defined as any strain that shows:
*diameter of the inhibition zone to Imipenem <22 mm and/or Ertapenem <21 mm
*Presence of discrete colonies in the inhibition zone of carbapenems
*MIC to carbapenems >2μg/ml or MIC to Ertapenem >0.5μg/ml
All CRE isolates were tested by phenotypic method: Modified Hodge test (MHT) and EDTA test.

RESULTS: During the study period, total of 1323 strains of Enterobacteriaceae were included.14 strains were resistant to carbapenem. The prevalence of CRE in our hospital is 0,01%. These included 8 resistant strains of Enterobacterspp, 4 strains of Klebsiella spp and 2 strains of Morganellamorganii. 15 strains were resistant to carbapenems by both disk-diffusion and MIC. None of the strains among the CRE isolates showed production of ESBL enzyme. 1 of the isolates (CRE) was MLB producer (EDTA+), 5 of these isolates showed positive result in MHT.

CONCLUSIONS: The emergence and the dissemination of CRE is an important threat to public health. CRE and, in particular, those with carbapenemases, are an emerging infection problem at the Blida university hospital center. Active monitoring is required to control their spread.

Clinical microbiology

Cod: 0430

SIMPLE AND EASY HEPATITIS C VIRUS (HCV) GENOTYPING USING HIGH RESOLUTION MELTING ANALYSIS (HRMA) TOGETHER WITH AMPLICON POST HCV VIRAL LOAD DETERMINATION

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BACKGROUND: Determinations of Hepatitis C virus (HCV) viral load and genotyping are important for therapeutic effect prediction of infected patient. For analysis of genotype, direct sequencing (DS) is the gold standard, but the operations are complicated and time consuming. The purpose of this study was to establish simple and easy HCV genotyping by high resolution melting analysis (HRMA) with non-labeled probes using amplicon of HCV viral load determination such as the ROCHE COBAS® Ampliprep/COBAS® TaqMan® HCV test (TM).

METHODS: TM amplicons of HCV infected patients were diluted 10,000 fold with PCR grade water for genotyping samples. HRMA with two non-labeled probes was performed by Light cycler®480 High Resolution Melting Master (ROCHE). Genotypes were validated by 5′ UTR sequences obtained by DS with 3130 genetic analyzer (Applied Biosystems).

RESULTS: Concentration of primers and probes were important factors in HRMA genotyping. Optimal forward primer, reverse primer, probe1 and probe2 concentrations were respectively 0.02, 0.2, 0.2 and 0.4 μM. HRMA with samples known genotype gave melting curves which were each characteristic of 1a, 1b, 2a and 2b. According to the melting curves with 59 TM amplicons, it was classified one case into genotype 1a, 26 cases into 1b, 13 cases into 2a and 15 cases into 2b. All of these 55 cases agreed with genotyping by DS. The other 4 cases, melting curve was not typical. Then DS classified to be respectively genotype 2a, 2, 3a and 6a. It takes only 2.5 hours to complete HRMA, much shorter than that for DS method (4hours).

CONCLUSIONS: HCV genotyping using non-labeled probes HRMA with TM amplicon can skip nucleic acid extraction and reverse transcription reaction, and is simple, rapid and easy to handle, which is useful as a routine test. In the cases with rare genotypes in Japan other than 1a, 1b, 2a and 2b, melting curve pattern is different from those for typical cases. DS is required in these cases for definite genotyping.

Clinical microbiology

Cod: 0431

MULTI–RESISTANT BACTERIA ISOLATED AT CANCEROUS CENTER OF BLIDA

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BACKGROUND: Cancer is the disease of the century. Patients with cancer constituted a fragile population to infections especially when these last are caused by bacteria multidrug-resistant bacteria (MDR). The aim of this study is to determine the rate of MDR bacteria causing infections in cancer patients at cancerous center of Blida (CAC).

METHODS: The study was a retrospective study over a period of 06 years, carried out in the laboratory of Microbiology, at the university hospital center of Blida, from 01/01/08 to 12/12/13. All the samples were processed, identified and antimicrobial susceptibility testing performed as per standard microbiological procedures. The Susceptibility to antibiotics was carried out according to recommendations of CLSI (Clinical Laboratory Standard Institute) by: disk diffusion method, search of ESBL (extended spectrum β-lactamase for all Enterobacteriaceae and non fermenting Gram negative Bacilli (NFR) potentially producing this enzyme, detection of methicillin-resistant Staphylococcus aureus MRSA and evaluation of the minimal inhibitory concentration (MIC) and the detection of carbapenem-resistant Enterobacteriaceae (CRE). The exploitation of the results was possible thanks to WHONET 5.6 software.

RESULTS: During the study period total of 531 strains of bacteria were isolated at CAC, 286 (53.86 %) strains of Enterobacteriaceae, 96 (18.08%) strains of NFR, 149 strains of Gram Positive Cocci (28.06%). The ESBL profile was detected at 28.32% of Enterobacteriaceae and 4.17% of NFR. 4 strains of Enterobacteriaceae and 5 NFR were resistant to carbapenem. MRSA profile was detected at 38.46% of staphylococcus among 78 implicated. Note the isolation of a strain resistant enterococcus to glycopeptide.

CONCLUSIONS: MDR Particularly common in immunocompromised patients, require the establishing of an effective preventive strategy in cancer centers.

Clinical microbiology

Cod: 0432

IMPACT OF PSEUDOMONAS AERUGINOSA BIOFILM FORMATION AND CARBAPENEM RESISTANCE ON CLINICAL COURSE OF DISEASE

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BACKGROUND: Despite adequate antimicrobial treatment, patients, with P. aeruginosa infection, mortality remains high. The aim of this study was to find out the impact of virulence factors of P. aeruginosa strains, isolated from different clinical material, on clinical course of disease.

METHODS: A retrospective patients with P. aeruginosa infection data analysis was performed. P. aeruginosa virulence factors – biofilm production, resistance to bactericidal serum activity and carbapenem resistance were tested. The tube method, described by Christensen, was used to detect biofilm formation. The ability of P. aeruginosa strains to resist to serum bactericidal effect was tested as described by Podschun R. The susceptibility testing was performed by the E-test method. Detected minimal inhibitory concentrations were evaluated according to EUCAST breakpoints. Proportions were compared using nonparametric statistical criterion chi-square or Fisher's exact test. Differences between groups were considered significant if $P<0.05$.

RESULTS: 33 (46.5%) of P. aeruginosa isolates were carbapenem-resistant, 37 (52.1%) of strains were multidrug resistant. All the P. aeruginosa strains isolated from the patients with trophic ulcers were carbapenem-sensitive (6 versus 0, P=0.033) and strains, isolated from burns were more often carbapenem-resistant (6 versus 1, P=0.016). Biofilm producers cause more often pneumonia then non-biofilm producers (76.2%, n=16 and 35.7%, n=5, respectively P=0.033). 68.4% (n=13) of non-biofilm producers were able to induce systematic response and 72.2% (n=13) of biofilm-producers were not able to induce systemic response in all strains, excluding respiratory tract cultures, respectively P=0.006. P. aeruginosa strains resistance to bactericidal serum activity had no impact on clinical course of infection. Lethal outcome was more often in age group ≥65 years, in patients with pneumonia diagnosis and those, who were hospitalized in intensive care unit.

CONCLUSIONS: In conclusion P. aeruginosa resistance to carbapenems is associated with resistance to other antimicrobial classes. P. aeruginosa biofilm production helps the strains to survive and is more important in chronic wound infections and in hospital acquired pneumonia cases.

Clinical microbiology

Cod: 0433

THE PREVALANCE OF THE ACINETOBACTER INFECTIOUS IN NEAR EAST UNIVERSITY: PERSPECTIVE OF THREE YEARS

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BACKGROUND: Acinetobacter spp is an catalase positive, oxidase negative, non-motile and non-fermentative Gram negative bacteria. Normal habitat are soils and waters in nature also Acinetobacter spp is a normal flora of the human body. Acinetobacter spp is an opportunistic pathogen especially for the hospitalized patients to cause serious infections, septic shock and death. That's the reason we must know the prevelance and antibiotics pattern of the Acinetobacter spp for the infections controls.

METHODS: All clinical samples were cultured in the 5% sheep blood agar and EMB media in microbiology laboratory. All the plates were incubated at 37 ^{o}C ,24-48 hours. For the identification and antibiotics pattern we used the Phoenix 100 system (Becton Dickinson, USA).

RESULTS: We documented Acinetobacter spp infections in Near East University Hospital reported between 2010-2013, retrospectively. Totally, 70 Acinetobacter spp infections determined. These were; Acinetobacter baumanii/calcoaceticus (n=55), Acinetobacter lwoffii/haemolyticus (n=2) ve Acinetobacter baumanii (n=13). Antibiotics pattern for the Acinetobacter baumanii/calcoaceticus were resistance to the Amikacin (74,5%); Ceftazidime (81,8%); Ciprofloxacıne (83,6%); Gentamicine (72,7%); Imipenem (78,13%); Levofloxacine (90%); Trimethoprim/Sulfamethoxazole (69%) and Piperacillin-Tazobactam (85,4%) but sensitive to the Ampicillin-Sulbactam (76,3%). For the Acinetobacter baumanii were sensitive to the Ampicillin-Sulbactam (%84,5) and most resistance to the Levofloxacine (70%) and Piperacillin-Tazobactam (% 70). Acinetobacter lwoffii/haemolyticus were sensitive to the all antibiotics.

CONCLUSIONS: It was indicated that antibiotics pattern of the Acinetobacter spp infections could be change between countrys infact between hospitals. Our results were similar to the prevalence of the Turkey. Unless there were no any prevalence studies for the Turkish Republic of North Cyprus (TRNC). So we could not compare our results.

Clinical microbiology

Cod: 0434

EVALUATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR DETECTION OF CAMPYLOBACTER JEJUNI

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BACKGROUND: Campylobacter jejuni is a leading cause of acute bacterial gastroenteritis in humans worldwide. A cultivation process is generally time-consuming requiring several days for diagnosis. Loop-mediated isothermal amplification (LAMP) assay is a simple and rapid technique that has been developed for detection of several infectious microorganisms. This study was evaluated a LAMP assay for C. jejuni detection using a set of 4 specific designed primers.

METHODS: To optimize LAMP conditions, various concentrations of $MgSO_4$, ($MgSO_4$: 4, 5, 6, 7, 8 mM; dNTP: 1.4 mM; betaine: 0.4 mM), dNTP (dNTP: 0.8, 1.0, 1.2, 1.4, 1.6 mM; $MgSO_4$: 6 mM; Betaine: 0.4 mM) and betaine (betaine: 0.1, 0.2, 0.3, 0.4, 0.5 mM; $MgSO_4$: 6 mM; dNTP: 1.0 mM) were determined respectively. An appropriate condition was used to evaluate specificity and sensitivity of the LAMP assay. DNA templates from fifteen bacterial genuses were tested for specificity and ten-fold serial dilutions of C. jejuni DNA were performed for sensitivity evaluation.

RESULTS: The result showed that optimization of 6 mM of $MgSO_4$, 1 mM of dNTP and 0.4 M of Betaine with temperature for amplification of 63^{o}C were the appropriate condition of LAMP assay. The LAMP preformed on DNA extracted from bacterial pure culture had a specificity of 100% and at least 5.6 pg or 1.6 x 10^6 copies of C. jejuni DNA could be detected using this LAMP assay.

CONCLUSIONS: The LAMP assay is faster and easier method for detection of Campylobacter with high specificity and sensitivity. The method may be appropriate for microbiological laboratory investigation of C. jejuni.

Clinical microbiology

Cod: 0435

OUTBREAK OF AN ARMA METHYLTRANSFERASE-PRODUCING ST39 KLEBSIELLA PNEUMONIAE CLONE IN A PEDIATRIC ALGERIAN HOSPITAL

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BACKGROUND: Here we report an outbreak of Klebsiella pneumoniae infections harboring extended spectrum b-lactamases (ESBL) and armA 16Sr RNA methylase that were detected in pediatric and neonatal intensive care units during the 2010 and 2011 surveys of 100 clinical strains of K. pneumoniae from Annaba hospitals in Algeria.

METHODS: Antibiotic susceptibility testing was performed using the disk diffusion method. Minimum inhibitory concentrations of three classes of antibiotics were determined using the E. test. Standard polymerase chain reaction amplification and sequencing were performed using primers targeting ESBL, 16S ribosomal RNA (rRNA) methyltransferases, aminoglycoside-modifying enzymes (AMEs), and quinolone encoding genes. Clonal relationships among the clinical isolates were performed using multilocus sequence typing.

RESULTS: From our clinical isolates, we found high rates of antimicrobial resistance that were linked to the presence of different ESBL encoding genes and AMEs, including 23 strains that harbored several ESBL encoding genes along with the 16S rRNA methyltransferase armA. Among these isolates, we identified a cluster of eight isolates of the ST39 clone between February and June 2010 in a pediatric ward, suggesting that an outbreak had occurred during this period.

CONCLUSIONS: The emergence of multidrug-resistant clones, which were likely responsible for a nosocomial outbreak, is worrying because there are already limited options in those critical situations. Finally, we believe that surveillance should be implemented to monitor the risk of emergence and spread of carbapenemases in Algeria.