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A multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB) for screening antimicrobial resistance genes

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Objective: Thousands of antimicrobial resistance genes have been reported and co-existence of multiple resistance genes in a single bacterial isolate is common. The multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB) is a convenient method for identifying up to 43 targets for 43 specimens. The aim of this work was to develop a rapid multiplexed assay to screen for resistance genes in *Enterobacteriaceae*.
Methods: Twenty-four resistance gene targets were chosen based on local epidemiological data, focusing on Gram-negative bacteria, particularly *Enterobacteriaceae*. Three major types of target genes were included: (1) those encoding aminoglycoside-modifying enzymes (*aac(3)-Ia*, *aac(3)-IIa*, *aac(6')-Ib/II*, *aac(6')-Ib*-cr, *aacA41* and *aadB*) or 16 s rRNA methylases (*armA* and *rtmC*); (2) those encoding beta-lactamases including ESBLs, carbapenemases and acquired AmpC enzymes (bla_{TEM}, bla_{SHV}, bla_{SHV-12}-like ESBL-type variants, bla_{CTX-M}, bla_{VEB}, bla_{OXA-1}-like, bla_{OXA-10}-like, bla_{OXA-23}-like, bla_{CMY-2}-like, bla_{DHA}, bla_{IMP}, bla_{VIM} and bla_{KPC}); (3) those mediating low-level resistance to quinolones (*qnrA*, *qnrB* and *qnrS*). Primers and probes were designed from aligned sequences and *in silico* evaluation was performed with BLAST. Additional probes were designed to allow bla_{SHV-12}-like variants and the *aac(6')-Ib*-cr variant to be identified. mPCR/RLB was carried as described previously. Control strains (n = 67) were obtained from local surveys or provided by colleagues in Australia or overseas. Single PCR for each target was carried for all controls to validate the mPCR/RLB assay, and bla_{SHV} and *aac(6')-Ib* amplicons were digested or sequenced.
Results: Twenty-two pairs of primers and 24 probes were designed and included in this assay. This assay correctly detected all target genes in all control strains, most of which contain multiple target genes (up to 8). The results of the mPCR/RLB assay completely matched those of individual PCR. This assay also correctly distinguished bla_{SHV-12}-like variants from other bla_{SHV} genes and *aac(6')-Ib*-cr from non-cr variants. No false positive nor false negative results were observed.
Conclusions: A mPCR/RLB assay able to detect 24 targets (resistance genes or groups of genes) was developed and validated. This assay proved highly accurate, and is likely to be a useful tool for the epidemiological surveillance of antimicrobial resistance genes in *Enterobacteriaceae*.

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The distribution of bla_{CTX-M} genes amongst clinically significant *Escherichia coli* isolates in 8 major hospitals in Kuwait

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Objective: Until recently, CTX-M bela-lactamases were virtually unknown in Kuwait. Literature evidence shows that CTX-M type enzymes are becoming the most prevalent extended-spectrum beta-lactamase (ESBL) in *Escherichia coli* with consequent increase in antibiotic resistance. The objective of this study was to investigate the epidemiology of CTX-M ESBL-producing *E. coli* among clinically significant isolates in all the 8 Kuwait governmental hospitals.
Methods: A total of 876 consecutive isolates were collected these hospitals; at least 100 per hospital. Production of ESBL was detected by the ESBL Etest method and confirmed by PCR. DNA extracts of ESBL-positive isolates were screened for the presence of bla_{CTX-M} gene using the following primers: MA-1 5′-SCS ATG TGC AGY ACC AGT AA-3′ and MA-2 5′-CCG CRA TAT GRT TGG TGG TG-3′. Strains with PCR amplicons positive for bla_{CTX-M} were sequenced using Applied Biosystems sequencer. The nucleotides were then analyzed with software available at website http://www.ncbi.nlm.nih.gov/blast. All the CTX-M-15 positive strains were evaluated for genetic relatedness using PFGE with *XbaI* digestion of the genomic DNA.
Results: Of the 876 isolates, 113 (12.9%) were ESBL producers, 88 (78%) of which produced CTX-M ESBL. Among these, CTX-M-15 was the most prevalent (84.1%), followed by CTXM-14 (6.8%), CTX-M-14b (5.7%) and TOHO-1 (3.4%). The highest number of *E. coli* carrying the bla_{CTX-M} gene was in Ibn Sina hospital (25.7%), a specialist hospital for immunocompromised patients, and the least in Maternity hospital (5.7%). CTX-M-15 was the predominant CTX-M type in all hospitals, representing 74 (84.1%) of the CTX-M ESBLs. Of these, 66 (89.2%) were found in isolates from the 3 predominant nationalities; Kuwaitis (36.5%), Egyptians (32.4%) and Indians (20.3%). No isolate was positive for this ESBL among the Saudi isolates. PFGE pattern of the CTX-M-15-positive isolates was heterogeneous.
Conclusions: Our data shows an explosive emergence of CTX-M-15 type ESBL that appears to be evenly distributed in *E. coli* strains isolated from patiens in the predominant nationalities residing in Kuwait and demonstrates no evidence of clonal spread.

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Antibiotic resistance in strains of *Streptococcus pneumoniae* encountered in Jamaica

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Objective: Antibiotic resistance in strains of *Streptococcus pneumoniae* varies widely in different parts of the world. There are very little published data on susceptibility of pneumococci encountered in countries in the Caribbean. In this report, we examine the susceptibility to 5 front-line antibiotics in 83 isolates encountered in 3 years (06–08) at the University Hospital of the West Indies (A Tertiary Care Teaching Hospital) in Kingston, Jamaica.
Methods: The isolates which were collected and stored in tryptic soy broth with glycerol at −70ºC were thawed in batches, pure cultures were obtained and again identified by routine methodology including Gram stain, optochin susceptibility and bile solubility tests. The MICs were determined by E test (AB Biodisk, Solna, Sweden) using *Streptococcus pneumoniae* ATCC 49619 as control and the results were interpreted according to the CLSI criteria. Only one isolate was included if there were more than one from the same patient.
Results: Thirty eight isolates (45.8%) were from sterile sites (mostly blood) and 45 (54.2%) from non-sterile sites (vast majority from sputum and ear swabs). Overall, 80.7% were susceptible to penicillin (MIC $\leqslant 0.06\,\mu g/ml$), 13.3% were intermediate (MIC $0.12–1\,\mu g/ml$) and 6% were resistant (MIC $\geqslant 2\,\mu g/ml$). Percent susceptibility to the other antibiotics were, erythromycin 86.7%, amoxicillin/clavulanate 97.6% and ceftriaxone 97.6%. All isolates were susceptible to vancomycin. Compared to data in many other countries, the levels of resistance in pneumococci continue to remain relatively low in Jamaica.
Conclusion: There are a large number of reports on antibiotic resistance in pneumococci from many developed countries. Published data from developing countries are few and far between. For a comprehensive global surveillance, there is a need to generate more such data from developing countries where *Streptococcus pneumoniae* continues to remain a major cause of morbidity and mortality.

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Surveillance from the Korean Antimicrobial Resistance Monitoring System (KARMS) for urinary tract infections in 2008

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Objective: Urinary tract infections (UTIs) are one of the most common infectious diseases diagnosed in community as well as in hospital. The objective of this study was to report the frequency of occurrence and antimicrobial resistance of uropathogens collected in South Korea in 2008 through the Korean Antimicrobial Resistance Monitoring System (KARMS) The system was established by Korea Centers for Disease Control and Prevention.

Methods: A total of 2,019 urine isolates from both outpatients and hospitalized patients were included.
Results: The patients' mean age was 46.7 years and most of the infections occurred among women (73.0%). Esherichia coli (58.2%) was the most frequent pathogen isolated followed by, *Enterococcus faecalis* (10.4%), *Klebsiella* spp. (4.6%), *Pseudomonas aeruginosa* (3.2%) and *Streptococcus agalactiae* (2.7%). Among the *E. coli* isolates, piperacillin/tazobactam, aztreonam, extended-spectrum cephalosporins, carbapenems and amikacin constitute reasonable therapeutic options for treatment of serious UTIs in South Korea (91.0–100.0% susceptible). High resistance rates to fluoroquinolones (21.4–26.8%) and trimethoprim/sulfamethoxazole (33.3%) were observed among the *E. coli*. In contrast, nitrofurantoin which is not available in South Korea displayed susceptibility rate of 91.7%. Against *Klebsiella* spp. infections, the only effective therapeutic option would be the carbapenems due to the high number of isolates producing extended-spectrum beta-lactamases (ESBL). Fluoroquinolones showed limited activity against *Klebsiella* spp. (60.2–76.5% susceptible) and the *P. aeruginosa* isolates showed high resistance rates to most antimicrobial agents tested.
Conclusion: Our results demonstrate that the uropathogens isolated in South Korea exhibit high resistance to various classes of antimicrobial agents. Fluoroquinolone-resistant *E. coli* and ESBL-producing *K. pneumoniae* constitute serious problem in South Korea.

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Methicillin resistance in clinical isolates of *Staphylococcus aureus* from University hospitals in Hamadan, Iran

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Objective: *Staphylococcus* areus is responsible for a wide variety of disorders; from mild skin to sever life threatening infections. Prevalence of methicillin resistant *S. aureus* (MRSA) increases progressively. The aim of this study was assessing antibiotic resistance in cases with positive cultures for staphylococcus aureus in education hospitals of Hamadan.
Method: In a 5-year period from 2003 to2007, all clinical isolates of *S. aureus* taken from blood samples or sterile secretions were included. Antibiotic susceptibility testing was performed with disc diffusion method. Required information was collected in questionnaires.
Results: In this study 263 clinical isolates of *S. aureus* were recognized, of which 95.1% were resistant to penicillin; and resistance to co-trimoxazole and ciprofloxacin was seen in 50.3% and 27.4% of cases respectively. MRSA consisted 73% of samples that mostly isolated from synovial fluid (100%) and trachea (89%). Among evaluated wards, the most MRSA isolates were observed from surgery ward (92%), and the least from infectious ward (46%).
Conclusion: According to obtained results and high prevalence of antibiotic resistance and especially high number of MRSA, appropriate use of anti-staphylococcal antibiotics is just essential. Also in sever staphylococcal infections it is better to choose antibiotic according to antibiogram.

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The incidence of side effects of linezolid and vancomycin in adult patients – a comparative study

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Objective: We have searched for the incidence of side and adverse effects of linezolid and vancomycin therapy in adult hospitalised patients.
Method: We have taken into study only cases with identified germ. Antibacterial therapy was performed according to the antibiogram. Only patients with at least 4 days of treatment with studied antibiotics were included into study. There were taken into study patients with severe infections treated with vancomycin (n = 255, 132 men and 123 women), hospitalised during 2000–2007. Median dose was 1 g bid (intravenously) and mean therapy duration was 8.41±2.46 days. The most frequently isolated bacteria were *Staphylococcus aureus* (123 cases), *Enterococcus* spp. (26 cases) and *Clostridium difficile* (34 cases). Another group of patients with severe infections was treated with linezolid (n = 197, 112 men and 85 women), hospitalised during 2004–2007. Median dose was 0.6 g bid and mean therapy duration was 10.82±3.58 days. The isolated bacteria were *Staphylococcus aureus* (103 cases), *Streptococcus pneumoniae* (45 cases), *Enterococcus* spp. (25 cases) and *Clostridium difficile* (24 cases). For each patient we performed physical examination and main laboratory analysis (biochemistry, electrocardiogram, complete blood count etc) at admittance and during the hospitalisation. No deaths have been reported during hospitalisation in studied patients.
Results: Main adverse reactions noticed for vancomycin were: skin rash (31 cases, 12.15%), abdominal pain (18 cases, 7.05%), vomiting (10 cases, 3.92%). Phlebitis was noticed in 84 cases (32.94%). Only 1 case manifested convulsions (0.39%) and anticonvulsive therapy was used. Serum bilirubine levels increased in 29 cases (11.37%). Main adverse reactions noticed for linezolid were: diarrhoea (12 cases, 6.09%), vomiting (8 cases, 4.06%), skin rash (5 cases, 2.53%). Also, psychomotor agitation was noticed in 7 cases (3.55%). 2 patients (1.01%) presented intense cough associated with prescribed antibacterial therapy.
Conclusions: Despite the noticed adverse reactions, replacement of the antibacterial drug was necessary in just 3 cases. The incidence of adverse reaction was significantly lower in case of linezolid vs. vancomycin, in the conditions of comparable efficacy.

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Clonal distribution of *Acinetobacter baumannii* in five hospitals in the Abu Dhabi Emirate

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Objective: The aim was to investigate the clonal distribution and antibiotic susceptibility of *A. baumannii* strains isolated in five hospitals in Abu Dhabi Emirate.
Methods: A total of 114 clinically significant, non repeat *A. baumannii* strains (43, 27, 26, 15 and 3 from Tawam, Mafraq, Sheik Khalifa, Al Ain and Rahba Hospitals, respectively) were collected between May and October 2008. Identification was confirmed by detecting the bla_{OXA51} gene by PCR with a multiplex system also targeting bla_{OXA23} and a Class I integron sequence. Assignment of strains to EU clones I, II or III was carried out by multiplex PCR amplifying allelic variants of bla_{OXA51}, csuE and ompA. Antibiotic susceptibility testing by disc diffusion and by micro-dilution were carried out according to CLSI standards.
Results: Thirty strains (26.3%) exhibited amplicon patterns consistent with EU clone I, 34 (29.8%) were typed as EU clone II, while 50 isolates (43.9%) were untypable. Eighty percent of the EU I isolates was non-susceptible to any of the beta lactams, fluroquinolones and aminoglycosides tested, while the respective figures for EU II and the untypable strains were 58.8% and 58.0%. The distribution of clones between hospitals was uneven: 46.7–46.7% of EU I strains were found in Tawam and Mafraq Hospitals, while Sheik Khalifa Medical Center had no EU clone I strain. This latter hospital yielded 52.9% of the EU clone II strains. 86.7% and 46.7% of the EU clone I strains carried bla_{OXA23} and the integrase, while the same figures for EU clone II isolates were 73.5% and 79.4%, and for the untypable ones 66.0% and 54.0%, respectively.
Conclusion: Multidrug resistant *A. baumannii* is an emerging threat in the hospitals of the United Arab Emirates, too. In the future active, nation-wide surveillance should follow the dynamics of the local epidemiology of this pathogen.