

Conclusions: Individuals with contact with foreign countries had high rates of intestinal colonization by Enterobacteriaceae producing CTX-M-types, especially those of the CTX-M-1 cluster. Our study points out the importance of ecological surveillance studies of faecal carriers of multi-drug resistance organisms to establish epidemiological measures that would allow controlling the dissemination of these organisms.

R2552 Detection of blaIMP-22 in a *Klebsiella pneumoniae* clinical isolate in an acute care hospital in Portugal

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Objectives: Carbapenem resistance in Enterobacteriaceae is one of the actual major therapeutic concerns. Routine procedures of the hospital clinical microbiology laboratory, alerted for the need of extensive study of some Enterobacteriaceae isolates in order to better understand the resistance profile, namely in isolates that showed reduced susceptibility or resistance to, at least, one type of carbapenem. With that purpose, isolates were selected for genotypic approach by PCR and sequencing.

Methods: In a set of 13 Enterobacteriaceae showing reduced susceptibility to carbapenems, collected since September 2010 till September 2011, a *Klebsiella pneumoniae* blood culture isolate, showed resistance to oxiiimino-beta-lactams, resistance to meropenem, reduced susceptibility to ertapenem, and susceptibility to imipenem, with MICs of 16, 4 and 2 µg/mL respectively, and susceptibility to aminoglycosides and fluoroquinolones. Identification and susceptibility testing were performed by Vitek2 (bioMérieux) and WalkAway (DadeBehring) systems. Total DNA of this *Klebsiella pneumoniae*, was subjected to amplification by multiplex PCR using primers for blaVIM, blaIMP and blaKPC and sequencing of the amplified fragment, using ABI-PRISM 3100 automatic genetic analyzer, showing the presence of blaIMP-22.

Results: In this study, we detected blaIMP-22, in a clinical isolate of *Klebsiella pneumoniae*. According to the CLSI guidelines, extended-spectrum β-lactamases production was not detected. Metallo-β-lactamases detection, by Etest MIC determination for imipenem and imipenem plus EDTA, was negative, highlighting the need of adequate phenotypic detection of this particular resistance threats. Genotypic determination of this resistance mechanism, seems useful in these situations of Imipenem susceptibility, masking carbapenemase production. This is an important issue to interpret hospital resistance epidemiology and to guide infection control procedures, to avoid outbreak installation.

Conclusions: As far as it seems, this is the first report of blaIMP-22 in a *Klebsiella pneumoniae* clinical isolate. The acquired metallo-β-lactamases represent a significant clinical threat due to their hydrolysis spectrum and infection control challenge. These atypical carbapenemase-producers may be overlooked in routine clinical microbiology laboratory testing, emphasizing the importance to survey and control the spread of such resistance determinants in nosocomial pathogens.

R2553 Characterisation of antibiotic resistance determinants in multidrug-resistant *Moraxella osloensis*, an opportunistic human pathogen

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Objectives: To characterise the antimicrobial resistance determinants present in *Moraxella osloensis*, isolated from human clinical trial studies.

Methods: Four *M. osloensis* isolates from the skin of two participants, one a placebo and one part of a clinical trial study undergoing antimicrobial treatment of Minocycline (EU ANTIRESEDEV Project) were characterized using an antimicrobial resistance (AMR) gene microarray chip (Clondiag TM GmbH). Phenotypic characterisations

were determined by antimicrobial disk diffusion assays and Phenotype Micro (PM) arrays from Biolog.

Results: Three of the four *M. osloensis* isolates from the skin trunk of human volunteers showed multiple resistance, both geno- and phenotypically to sulphonamide, beta- lactamase and tetracycline and produced positive signals for antibiotic resistance genes sul2, sul3, blaCMY, blaPER, blaOXA2 and tet37 with the microarray. The fourth strain was sensitive to all antibiotics tested and did not produce any positive signals with the AMR gene array. Biochemical characteristics determined using the PM arrays showed differences between the AMR resistance and sensitive strains.

Conclusion: *Moraxella osloensis* is an opportunistic human pathogen known to cause diseases such as endocarditis, osteomyelitis, septic arthritis, catheter infection and meningitis. At present all diseases have been treatable and currently there is no record in the literature of *M. osloensis* with multiple antibiotic resistances. The present discovery of multiple drug resistance in isolates of human origin is worrying and follows a growing trend of multiple resistances acquired in bacterial pathogens.

R2554 Screening of antibiotic-resistant Gram-negative organisms in hospital settings from Bucharest, Romania

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The purpose of this study was to establish the main resistance phenotypes and their genetic determinants in Gram-negative bacilli (GNB) isolated from intensive care units (ICU).

Material and Methods: A number of 531 GNB strains (334 Enterobacteriaceae and 197 Pseudomonadaceae) were isolated from 1166 positive clinical samples collected from patients hospitalized during 2011, in the ICUs of two big hospital from Bucharest, Romania. Their resistance phenotypes were established using: disk diffusion test, double-disk diffusion test (DDST) with amoxicillin-clavulanic acid (AMC), cefotaxime (CTX) and ceftazidime (CAZ), DDST with AMC plus EDTA, imipenem (IPM) and IMP plus EDTA, Modified Hodge Test (MHT) and E-test ESBL, MBL and AmpC. The genetic support of the antibiotic resistance was investigated by simple and multiplex PCR reactions for class A Ser- β-lactamases (PSE, CARB, TEM genes families), class B – metallo-β-lactamases -MBL (IMP, VIM, SPM gene families), class C – AmpC and ESBL, as well as ciprofloxacin resistance genes (gyrA, parC) as well as the presence of mexB, mexD, mexF and mexY genes, encoding for multi-drug efflux pumps. In accordance with the recommended definitions of the degree of multidrug resistance, 32% of the GNB exhibited a multi-drug resistance (MDR) phenotype (*Escherichia* sp., *Klebsiella* sp., *Serratia* sp., *Acinetobacter* sp., *Pseudomonas* sp.), 13.2% were extended-drug resistant (XDR) (*Klebsiella* sp., *Acinetobacter* sp.) and 5.6% pan-drug resistant (PDR) (*Enterobacter* sp. and *Klebsiella* sp.). The gyrA gene, as well as the mexB, mexD, mexF si mexY genes, encoding for the efflux pumps mexAB-oprM, mexEF-oprN10 si mexCD-oprJ were detected in ciprofloxacin resistant strains and also correlated with the phenotypic resistance to aminoglycosides and carbapenems. The ESBL phenotype was correlated with the presence of blaSHV, blaTEM and blaPSE genes, while the MBL phenotype with the presence of blaVIM gene.

Conclusion: The increasing resistance in GNR provides an important signal that we need to improve our understanding of the genetic and biochemical basis of resistance mechanisms in the bacterial strains circulating in our geographical area, by using phenotypic and resistance genotyping tools.