P1007
Poster Session IV
Molecular epidemiology of MDR Enterobacteriaceae

INVESTIGATING ANTIMICROBIAL RESISTANCE IN SOIL CULTURABLE GRAM NEGATIVE BACTERIA

D. Jones-Dias[1], E. Ferreira[1], J. Almeida[1], M. CaniÁa[1]

[1]Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

Many calls have been made regarding resistome investigation but only a small number of studies were focused on the role of environmental antibiotic resistance. The soil may be a source of mobile antibiotic resistance genes, but this knowledge needs to be assessed extensively, directing this investigation to resistance mechanisms that are relevant in the clinical setting, such as the production of β-lactamases, which constitutes the main antimicrobial resistance mechanism in clinical Gram negative bacteria. This work aimed to assess the soil resistome and determine whether different agricultural practices influenced the diversity of antibiotic resistant isolates and mechanisms.

Nine soil samples were collected from intensive (n=3), extensive (n=3), and organic (n=3) agricultural settings, in July 2012 and February 2013, covering the two main seasons; GPS coordinates, temperature and humidity values were recorded for all sampling sites. Then, soil samples were incubated in Brain Heart Infusion (BHI) enrichment broth followed by selection of resistant Gram negative bacteria in MacConkey agar plates containing 2µg/µl of cefotaxime (CTX), imipenem (IMP) or trimethoprim (SXT). The isolates were then identified through 16S rRNA universal sequencing or API20E/NE and evaluated in what regards their antimicrobial susceptibility by standard disk diffusion method against 32 antibiotics from different classes, according to the non specific French Society of Microbiology guidelines. The molecular screening for the main mobile β-lactam resistance genes (Ambler class A, B, C, D β-lactamase-encoding genes) and genetic elements (class 1, 2 and 3 integrons) was performed by PCR and further sequencing.

Overall, we collected 232 Gram negative resistant isolates distributed mainly among *Achromobacter*, *Ochrobactrum*, *Pseudomonas*, *Stenotrophomonas*, *Enterobacteriaceae* groups. Among those, 27.5% of the antibiotic resistant (CTX, IMP SXT) isolates were recovered from organic agriculture, 26.3% from extensive agriculture and 25.4% from intensive agriculture. The great majority of the isolates gathered were multidrug resistant (85.8%) among which β-lactam antibiotics, cloramphenicol, nitrofurantoin and fosfomycin were the main contributors. These isolates were mostly susceptible to aminoglycosides (83.6%), specifically amikacin (95.7%). The presence of synergy between β-lactam antibiotics and β-lactamase inhibitors frequently suggested the production of metallo β-lactamases, AmpC but also extended-spectrum β-lactamases. The molecular screening detected the occurrence of mobile resistance genes and genetic elements such as bla_{TEM}, *dfrA*, *aadA* and class 1 integrons in *Enterobacteriaceae* and *Pseudomonadaceae* isolates.

These results gathered so far showed a high level of multidrug resistance in soil bacteria, independent of the anthropogenic actions directly related to soil use. The report of both antibiotic resistance and mobile genetic elements highlights the spread of resistance genes by mobile genetic elements and the possibility of shared resistance genes between environmental and clinical bacteria. Indeed, the evaluation of environmental resistant bacteria is an important assignment that continuously contributes to the anticipation of emergent resistant pathogens.