Conclusion: The study led to identification of a number of transcripts showing over-expression in drug resistant isolates, some of which corresponded to known genes like PSA-2, nucleoside transporter (NT), tetracycline resistant protein homologue (TRH) and proteasome regulatory ATPases. Three clones corresponded to the PSA-2, a glycoprotein with leucine rich repeats that has a role in facilitating attachment and internalization of parasites by macrophages, resisting complement mediated cell lysis. NT and TRH may promote resistance to drug by inhibiting the drug intake by the parasite. Proteasome and associated ATPases help increased survival of Mycobacterium inside macrophage by lending resistance to nitric oxide. The over-expression of this protein in drug resistant Leishmania may help the parasite to persist in host. The study establishes the utility of genome-wide RNA expression profiling in Leishmania and identifies numerous genes with potential role in drug resistance.

Emergence and molecular evolution of antibiotic resistance

O205

A multidimensional screening of non-clinical isolates reveals metallo-beta-lactamases in the environment

S. Quinteira, H. Ferreira, L.V. Peixe *(Porto, P)*

Objectives: Even though increasingly reported in many countries, metallo-beta-lactamase (MBL)-producing Gram-negative bacilli have been associated to the hospital setting, suggesting a relative confinement within this ecological niche. In this study, the presence of MBLs and imipenem susceptibility in Gram-negative bacilli were evaluated among non-clinical sources in order to verify a possible spreading into new, previously undescribed, environments.
Methods: Samples (24 from healthy human volunteer faeces, 30 from poultry skin, 4 from swine faeces, 13 from rivers and 29 from hospital wastewaters-receiving urban sewage) were collected in Northern Portugal between 2001 and 2004. Sample aliquots were platted, in MacConkey agar and/or Pyocyanosel agar supplemented with imipenem (2 mg/L) and colonies with different morphology were collected. Species identification was performed with the API 32GN. Imipenem susceptibility was determined using the disk diffusion method. A bioassay method was employed in order to detect MBL production and positive results were later confirmed by a multiplex PCR assay designed to amplify both blaVIM and blaIMP genes. Characterisation of MBL-type was possible through sequencing.
Results: 190 Gram-negative bacilli (21 lactose-fermenters and 169 non lactose-fermenters) were isolated from different sources. Decreased susceptibility to imipenem was observed in 64 isolates from sewage (n = 25), rivers (n = 17), swines (n = 12) and healthy volunteers (n = 10). All poultry isolates showed imipenem susceptibility. Positive bioassays were detected in 49 out of the 64 isolates with reduced susceptibility to imipenem. blaVIM-2 gene was detected in three isolates: two Pseudomonas aeruginosa (river and hospital sewage) and a *P. alcaligenes* (hospital sewage). blaIMP was not detected in none of the isolates.
Conclusions: Carbapenems are in many cases the last therapeutic resort and the detection of carbapenem-hydrolyzing MBL producers in the hospital setting is a worrisome phenomenon. Although VIM and IMP-producing strains were not observed in animals and human healthy volunteers, these enzymes seem not to be restricted to the hospital setting anymore. Disturbingly, the results emerging from this study show that, even if in very low frequencies, the presence of VIM-2 loose in the environment, namely in sewage and rivers, may undermine the effectiveness of carbapenems, largely depending on the eradication or confinement of resistant bacteria.

O206

CTX-M-producing *Escherichia coli* now the dominant cephalosporin-resistant Enterobacteriaceae in London and SE England

N. Potz, R. Hope, M. Warner, A.P. Johnson, D. Livermore *on behalf of the London & South East ESBL Project Group*

Objectives: There is increasing concern about the spread of Enterobacteriaceae, principally *E. coli*, producing CTX-M-type extended-spectrum beta-lactamases (ESBLs) in the UK. This is reflected in increasing surveillance reports and submissions to the reference laboratories, with many isolates reportedly from community patients. To better assess the situation, a prospective survey of cephalosporin-resistance was undertaken for Enterobacteriaceae across London and Southeast England.
Methods: Eighteen laboratories participated: 10 in London and 8 elsewhere in southeast England. These screened all Enterobacteriaceae isolates using cefpodoxime or both cefotaxime and ceftazidime. Isolates resistant to any of these 3 cephalosporins were submitted for centralised susceptibility testing and identification. Interpretive reading was performed, allowing an inference of resistance mechanism(s).
Results: During a 12–week collection period, >1000 cephalosporin-resistant *Enterobacteriaceae* isolates were collected, mostly submitted on the basis of their cefpodoxime resistance. *E. coli* was by far the most numerous species (>50% of all isolates), with over 70% of these having an ESBL, usually conferring a CTX-M phenotype. *Klebsiella* and *Enterobacter* spp. were also well represented (each over 15% of resistant isolates). The majority of the resistant Klebsiella isolates had phenotypes indicative of ESBL production but two-thirds of enterobacters had phenotypes implying derepression of AmpC. ESBLs were detected in a few *Citrobacter*, *Morganella* and *Serratia* spp. isolates, but not in *P. mirabilis*. Approximately 60% of all cephalosporin-resistant Enterobacteriaceae were inferred to be ESBL-producers, mostly with CTX-M phenotypes, whereas isolates with AmpC phenotypes accounted for 20%. The remaining 20% comprised of isolates inferred to have other beta-lactamases, or non-enzymatic resistance.
Conclusion: Until 2000 CTX-M-beta-lactamases were unknown in the UK and cephalosporin-resistant *Enterobacteriaceae* were mostly enterobacters with AmpC or Klebsiella spp. with TEM or SHV ESBLs. This survey shows a dramatic shift towards *E. coli* as the main host for ESBLs and cephalosporin resistance, and towards CTX-M phenotypes.