

tobramycin (PA: 91% vs. NPA: 34%; $p < 0.0001$), and amikacin (98% vs. 67%; $p < 0.0001$), while for other (combinations of) drugs tested no difference was found.

Conclusions: Since PA ESBL genes had the same prevalence among ESBL positive *E. coli* in CA and nosocomial UTIs, existence of two different compartments for ESBLs was not supported. Compared to *E. coli* with a NPA ESBL gene, *E. coli* with a PA ESBL gene were more susceptible to ciprofloxacin and aminoglycosides. Presence of PA ESBL genes could not be predicted upon susceptibility profiles.

P1643 Presence of extended-spectrum beta-lactamase producing Enterobacteriaceae in wastewaters, Kinshasa, the Democratic Republic of the Congo

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Objective: Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae are a public health concern worldwide, but few data are available from Central Africa. The aim of this study was to assess the presence of ESBL-producing Enterobacteriaceae in wastewaters of Kinshasa, capital of the Democratic Republic of the Congo (DRC).

Method: Enterobacteriaceae were recovered from environmental water samples and screened for ESBL production by disk diffusion using cefotaxime, ceftriaxone and ceftazidime disks. Confirmation of ESBL production was done by the combined double-disk method using cefotaxime and ceftazidime alone and in combination with clavulanic acid, according to CLSI guidelines (CLSI M100-S21). Double-disk confirmed ESBL producers were further identified up to species level and tested for antimicrobial susceptibility using Microscan NBC42 panels. Detection and identification of ESBL producing bla genes was performed by a commercial multiplex ligation PCR microarray Check-MDR CT101.

Results: In February 2011, water was sampled at 11 sewer and nine river sites in nine municipalities (both residential quarters and slums) of the city of Kinshasa. A total of 194 non-duplicate Enterobacteriaceae were recovered. Eighteen isolates were positive for ESBL screening, of which 14 (7.2% out of 194) were confirmed by disk diffusion. They were recovered from eight sampling sites (five sewers and three rivers) in five different municipalities. The main species were *Enterobacter* spp. (46.6%) and *Klebsiella pneumoniae* (40.0%). Co-resistance to both aminoglycoside and fluoroquinolone antibiotics was observed in 10 isolates, the remaining isolates showed co-resistance to either aminoglycoside ($n = 3$) or fluoroquinolone antibiotics ($n = 1$) respectively. All but one isolates carried blaCTX-M genes belonging to CTX-M1 group. For one isolate, no putative bla gene was detected.

Conclusion: A recent study from India showed tap water samples to be contaminated with carbapenemase bla NDM-1 producing organisms. The present results demonstrate that multiresistant bacteria are contaminating wastewater systems. This finding suggests a widespread dissemination of ESBL producing bacteria in the community of Kinshasa. Cities in Central Africa should be added to the map of potentially ESBL-contaminated environments.

P1644 Zoonotic potential of multidrug-resistant *Escherichia coli* clonal groups in Portugal

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Objectives: Multidrug-resistant (MDR) plasmids together with clonal dissemination of resistant isolates are possibly the most successful combination of factors contributing to the spread of antibiotic resistance genes. Our aim was to identify clones associated with multidrug-resistance among human and dolphin isolates, in order to evaluate zoonotic potential and risk.

Methods: Sixty two MDR human *Escherichia coli* isolates were randomly selected from NIH collection, being previously isolated from different clinical specimens in seven geographically apart Portuguese hospitals from 2004 to 2009. Two *E. coli* isolated from dolphin's respiratory exudates in 2009 and 2010, at the National Laboratory of Veterinary Research, were also included in this study for their zoonotic potential analysis. Antimicrobial susceptibility was performed by broth-microdilution method (EUCAST). PCR and sequencing were used to screen and identify beta-lactamase and Aac(6')-Ib-cr encoding genes, while PCR-based replicon typing was used to characterize plasmids from MDR isolates. Genetic relatedness of human and dolphin isolates was examined both by PFGE and MLST. Mobile genetic elements were also investigated through PCR mapping assays.

Results: Regarding the human isolates, 48 (77%) were CTX-M producers. We detected blaCTX-M-1 ($n = 4$), blaCTX-M-3 ($n = 3$), blaCTX-M-14, blaCTX-M-15 ($n = 34$), blaCTX-M-32 ($n = 3$), ($n = 4$), blaTEM-1 ($n = 39$), and blaSHV-12 ($n = 8$) genes as well as aac(6')-Ib-cr ($n = 26$). Concerning the isolates recovered from the dolphins, one of them produced TEM-1, OXA-30, CTX-M-15 and Aac(6')-Ib-cr and the other TEM-1, Aac(6')-Ib and Aac(6')-Ib-cr. Replicon-typing revealed a severe predominance of IncF plasmids in both animal and human isolates; IS26 and ISEcp1 were also detected in both groups, being associated with blaCTX-M-15 and Aac(6')-Ib-cr plus OXA-30, respectively, in one of the dolphin isolates. Genetic relatedness analysis by PFGE revealed one major cluster corresponding to a single epidemic clone A, which included 22 (35%) of all human isolates and both dolphin isolates. They exhibited the same combination of MLST alleles, corresponding to ST131.

Conclusion: This study illustrated the dominance of common antibiotic resistance genes, plasmids and clonal groups, specifically blaCTX-M-15, aac(6')-Ib-cr, IncF plasmids and ST131 in both human and animal isolates, reflecting their linkage and enhancing their zoonotic potential. Studies should be performed to further deepen their role as hotspots of resistance.

Automation of the microbiology laboratory

P1645 Evaluation of manual vs. automated plate spreading techniques

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Objective: A new techniques which has very recently been introduced within the microbiology laboratory is the idea of automated plate spreading techniques aimed at producing more single colonies than the original manual method of plate spreading, reducing time from the sample being taken to results and treatment, and producing more standardised results for a more accurate measure of bacterial growth on the agar plate. For this study the Inoqula[®] from Kiestra[®] was used which demonstrates a unique bead rolling technology used in the acquisition of single colonies.

Methods: For the acquisition of single colonies five dilutions to McFarland standard of an ATCC strain of *Staphylococcus aureus* were inoculated onto blood agar plates and streaked using a sterile loop and the Inoqula from Kiestra and single colonies counted, a selection of patient samples were also used. The time trials were conducted with pre-prepared batches of a set number of specimens. Faeces samples were also used, the manual plates were inoculated straight from the sample the automated method used maximum recovery diluents (MRD) approximately 1 g a pea sized lump was inoculated into the MRD and left to stand at room temperature for an hour. Because of the nature of MRD a second study using faeces samples was also conducted using samples known to contain a pathogen but also contain a lot of normal gut flora to see how long to you could leave the MRD before the normal gut flora is too over grown.

Results: As the concentration of bacteria increased the numbers of single colonies fell, the coefficient of variation for each McFarland standard concentration was considerably lower for the automated method. During the time trial the automated method showed to be up to