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Antibiotic resistance and genomic phylogenetic analysis of animal pseudomonad isolates in comparison with human isolates

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Background: Antibiotic resistance (ABR), a typical emerging phenomenon of highly complex and self-organising systems evolving at the edge of chaos, is a worldwide problem.

Beside over consumption and inappropriate use several fundamental issues are still not well understood, like their natural role, while data on incidence and prevalence of antibiotic resistances among animals, domesticated and wild are also only fragmentary available.

Methods & Materials: Our batch (86) included pseudomonad isolates, mainly aeruginosa, (of 50 pets (dog, cat, turtle, parrot), 16 farm animals (cow, sheep, horse, pig, goat), 7 zoo animals (seal, dolphin, kangaroo, tamarind) and 13 wild sea turtles.

The strains were biochemically (Vitek-BioMérieux) and genomically identified (pseudomonad-specific *opr*I/L lipoprotein PCR) before antibiotic resistance profiling and *P.aeruginosa* serotyping was done. Antibiotics tested were temocillin, ticarcillin, ticarcillin + clavulanic acid, piperacillin/tazobactam, ceftazidime, cefepim, aztreonam, imipenem, meropenem, amykacin, gentamycin, tobramycin, ciprofloxacin, tigecyclin, fosfomycin, colistin, and timethoprim + sulfamethoxazole. All strains were genotyped by Rep-PCR (Diversilab-BioMérieux). All isolates originated from Portugal except the turtle isolates who were sampled in Sao Tomé.

Results: Serotypes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 15 were detected while 6 was the most common and 5 and 7 only present among wild turtles. Most prevalent serotype among dogs (39) was 1 and 6 (23 and 20%), among farm animals serotype 1, 4 and 6 (>50%) were detected, serotypes 1, 6, 9,11 were detected among the zoo isolates. Multidrug-resistant bacteria was isolated among pets (20%) and farm animals (13%) while none was found among zoo animals but surprisingly 2/13 sea turtle showed resistance for' ticarcillin + clavulanic acid, aztreonam and fosfomycin'. One extensively drug-resistant (XDR) isolate, only sensitive for aminoglycosides and colistine, while intermediate for cephalosporin, was isolated from a dog. Serotype was 12, a serotype associated with a human XDR clonal-cluster typically found in intensive care units. Resistance levels ranged from 98% (temocillin) to 0% (ceftazidime, cefepim, amikacin, colistin). Genotyping did not show any animal specific cluster. All isolates were homogeneously scattered in the global *P.aeruginosa* population structure.

Conclusion: Those data show the presence of ABR among *P.aeruginosa* isolates, originating from domesticated as well as wild animals.

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Serotype prevalence and antibiotic resistance among adult invasive *Streptococcus pneumoniae* isolates in Turkey, 2005-2011

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Background: *Streptococcus pneumoniae* infections are major health problems because of the virulence of this bacteria and its ability to develop resistance. Surveillance systems are necessary to monitor the burden of pneumococcal disease, especially in the setting of pneumococcal vaccination programs. Aim of this study is to evaluate the serotype distribution and antimicrobial suscestibility of *Streptocccus pneumoniae* causing invasive pneumococcal disease in adults (>18) during the time period before the 13-valent conjugated pneumococcal vaccine introduce in Turkey.

Methods & Materials: *Streptocccus pneumoniae* strains were collected from 8 different centre between 2005 and 2011. All collected strains were frozen and kept at -80 C, in glycerol before used. Strains were identified by screening for alpha hemolysis, optochin susceptibility, sodium deoxycholate lysis, and latex tests to detect S. pneumoniae antigen. *S.pneumoniae* strains were studied for penicillin, cefotaxime, erythromycin and moxifloxacin susceptibilities by E-test (AB Biodisk, Sweden). Results were evaluated according to the CLSI standards and the strains isolated from CSF from others were seperated, for the interpretation of penicilin and cefotaxime. Serogrouping was performed with the latex particle agglutination and serotyping was made with the conventional Quellung reaction using commercial type-spesific antisera.

Results: In this study, 176 *Streptococcus pneumoniae* clinical isolates were tested for antimicrobial susceptibility and serotyping. These isolates were sampled from: blood cultures(n:109); cerebrospinal fluid(n:26); bronchoalveolar lavage(n:21) and the other sterile body fluids(n:20). Penicillin resistance in *S.pneumoniae* iso-

lates were found to be 23.3% (41/176) and intermediate resistance rate was 14.2% (25/176) for oral penicillin. Penicillin susceptibility testing, yielded a 43% overall resistance to penicillin with 42.3% of strains isolated from CSF(meningitis), and only 0.7% in other samples. Resistance to cefotaxime which were isolated from CSF was 3.8% and from non-meningitis was 2%. Erythromycin resistance was detected as 25%. No resistance was detected to moxifloxacin. The most common S.pneumoniae serotypes were determined as serotype 3(13.6%), 19A(9.1%), 19F(8%) and 6B(5.1%). Serotypes 19A and 19F exibited higher rates of penicilin and erythromycin resistance. The coverage rate for 13-valent conjugated vaccine is 49.4%.

Conclusion: Vaccination with 13-valent conjugated vaccine seems to be appropriate for adults in our country.

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Detection and identification of carbapenemase types in enterobacteriaceae isolates from blood cultures

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Background: The emergence of carbapenem resistance has been increasingly reported amongst Enterobacteriaceae and represents a major clinical concern. In this study we aim to determine carbapenemase existance and types in carbapenem resistant *Enterobacteriaceae* isolates from blood cultures.

Methods & Materials: We studied *Enterobacteriaceae* strains isolated from blood cultures in the Cerrahpasa Faculty of Medicine, medical microbiology laboratories during the 12 month period from March 2011 to May 2012. Antibiotic susceptibilities were determined by the disk diffusion method according to the CLSI guidelines. All isolates that showed an inhibition zone of < 23 mm to ertapenem disk get further investigation. The MICs of ertapenem, imipenem, meropenem were determined using E-test strips. PCR and sequencing were used to determine the for the VIM, IMP, KPC, OXA-48 and NDM encoding genes.

Results: Reduced susceptibility to ertapenem detected using disk diffusion in total 37 isolates. Among these bacteria, MIC_{90} values for ertapenem, imipenem, meropenem were > 32 μg/ml. bla OXA-48 was detected in 19 of 26 isolates of *Klebsiella* spp., 2 of 7 isolates of *Escherichia coli*, 3 of 4 isolates of *Enterobacter* spp. and VIM-5 was found in one *Klebsiella* spp. isolate. No IMP, NDM and KPC encoding genes were found among the isolates.

Conclusion: In conclusion, ertapenem disk diffusion test appears to have the highest sensitivity for screening. In our study, OXA-48 accounted for the most frequent carbapenemase-encoding gene. OXA-48 type carbapenemases are highly prevalent in our hospital setting. Determination of enzymes leading to carbapenem resistance in local and country level and providing epidemiological data contribute to rational use of carbapenemases used for life-threatening infections.

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Genetic detection of β-lactamase genes in *Klebsiella pneumoniae* and *Escherichia coli* isolates from wastewater

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Background: ESBLs are enzymes responsible for bacterial resistance to penicillins, aztreonam and cephalosporins and have been detected in several members of the *Enterobacteriaceae* family, commonly in *Klebsiella pneumoniae* and *Escherichia coli*. Both bacteria are commensals but are also opportunistic pathogens. Resistant bacteria are commonly found in wastewater treatment plants (WWTPs), where if decontamination fails, resistant bacteria may spread into the environment. The aim was to identify β-lactamases from resistant *K. pneumoniae* and *E. coli* isolated from wastewater samples.

Methods & Materials: Wastewater was collected in winter from three WWTPs. Samples were cultured on chromID ESBL media plates and colonies were identified using the MALDI-TOF MS system. Antibiotic susceptibility profiles were determined for confirmed isolates using the VITEK®2 automated system. Genomic DNA was extracted from the isolates and used in PCR assays.

Results: A total of six *K. pneumoniae* and 38 *E. coli* were identified from the influent and anaerobic zones of the WWTPs and had the ESBL-producing profile according to the MALDI-TOF MS and the VITEK®2 systems, respectively. For the *K. pneumoniae* isolates, 100% (6/6), 67% (4/6), 100% (6/6), 83% (5/6) and 67% (4/6) were positive for TEM, SHV, CTX-M group I, OXA-1-like and OXA-48-like detection, respectively. For the *E. coli* isolates, 100% (38/38), 21% (8/38), 79% (30/38), 13% (5/38), 37% (14/38), 55% (21/38) and 63% (24/38) were positive for TEM, SHV, CTX-M group I, CTX-M group III, CTX-M group IV, OXA-1-like and OXA-48-like detection, respectively. One isolate was positive for CTX-M group I and group III co-detection, while two isolates were positive for CTX-M group III and group IV co-detection. All isolates were negative for KPC and NDM.

Conclusion: Although several ESBL-producing *K. pneumoniae* and *E. coli* isolates were identified harbouring various β-lactamase genes, no isolates were identified from the effluent zones of the WWTPs, suggesting a functional system; however, a glitch in the treatment process may present a dissemination route of antibiotic resistant bacteria from WWTPs to the environment. Any dissemination route is worrisome as this will threaten public safety especially in cases where immunocompromised individuals may use the potentially infected water.

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