

degree of the resistance to tested antibiotics. Strains were susceptible only to amikacin (MIC = 8 mg/l) and colistin (MIC = 1 mg/l).

Conclusions: Despite all neighbouring countries, MBLs have never been described and detected in the Czech Republic before this finding. In this time, it is not possible to identify a geographical origin of these isolates. However, the IMP-7 MBL, firstly described in Canada recently in Malaysia and Japan, seems to be uncommon in the Europe. Epidemiological data will be completed by MLST which is on going. This work was supported by a research project grant MSM 2E08003.

P1496 **Multiresistant epidemic clones of *Pseudomonas aeruginosa* in the Czech Republic**

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Objective: To determine whether the high prevalence of antimicrobial resistance among bloodstream isolates of *Pseudomonas aeruginosa* in the Czech Republic is associated with the clonal spread of multidrug resistant (MDR) strains.

Methods: The study set included 108 bloodstream isolates, which were selected from 437 isolates of *P. aeruginosa* collected in the Czech Republic within the European Antimicrobial Resistance Surveillance System (EARSS) project in 2007. The 108 isolates originated from 49 hospitals in 36 cities. They were tested for susceptibility to piperacillin, ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, gentamicin, tobramycin, amikacin and colistin by E-test. The genotypes of the isolates were assessed by multilocus sequence typing (MLST), macrorestriction analysis of genomic DNA and class 1 integron typing.

Results: Forty-six isolates were susceptible to all antimicrobial agents while 16 and 46 isolates were resistant or intermediate to 1–3 and 4–9 agents, respectively. A total of 41 multilocus sequence types (ST) were identified, which, except for four unique STs, differed from each other in at least three alleles. ST235 and ST175 included 19 and 16 isolates, respectively. The isolates with either ST235 or ST175 originated from 25 hospitals in 19 cities. Each of other eight STs included 3–7 isolates, seven STs were found in 2 isolates and the remaining 24 isolates yielded each a unique ST. Isolates of the same ST had highly similar macrorestriction profiles. ST235 and ST175 encompassed 34 (74%) of 46 isolates resistant to more than 3 agents. Class 1 integrons were found in 47 MDR isolates, with at least 18 different integron variable regions. Twelve isolates with ST235 harboured an integron with a 1.9 kb variable region while 15 isolates with ST175 shared an integron with a 1.6 kb variable region.

Conclusion: The high prevalence of antimicrobial resistance in *P. aeruginosa* isolates in the Czech Republic is predominantly associated with two MDR epidemic clones, one of which (ST235) belongs to international clonal complex CC11.

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P1497 **Isolation and characterisation of an imported pan-resistant *Pseudomonas aeruginosa* clinical isolate producing three different ESBL enzymes, hyperproducing multidrug-efflux pumps**

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Objectives: *Pseudomonas aeruginosa* is one of the most frequently isolated nosocomial pathogens, causing life-threatening infections, such as pneumonia, bacteraemia, and wound infections. It exhibits intrinsic resistance to several β -lactams and acquire easily additional resistance mechanisms, including the production of extended spectrum β -lactamases (ESBLs), down-regulation of porins, hyperproducing multidrug efflux-pumps, confer resistance to almost all antipseudomonas antibiotics.

Methods: During the period of 2004 to 2008 we isolated 27 ceftazidime resistant, non-mucoid *P. aeruginosa* isolates from different non-cystic

fibrosis patients, hospitalised in nine different hospital wards of South-Hungary. Identification by VITEK 2 system and susceptibility test by disk-diffusion method was performed, using CLSI breakpoints. The genes of the extended spectrum β -lactamases (PER-1, PER-2, TEM, SHV, GES, VEB-1, OXA groups) were looked for by PCR methods. The iso-electric focusing of the β -lactamases was performed, the enzymes were visualised with nitrocefin. To investigate the location of the β -lactamase gene plasmid purification, PCR detection of the Tn1213 specific IS element were performed. The transcription of the chromosomal genes encoding the OprD, ampC, and the efflux pumps MexAB-OprM, MexXY-OprM, MexCD-OprJ was studied with real-time PCR assays. The genetic relatedness of the strains was investigated by PFGE and MLST analyses.

Results: PCR experiments revealed the presence of blaPER, blaOXA-I, II group in one isolate. Sequencing of the coding region and the RFLP analyses identified the PER-1, OXA-2 and OXA-74 genes. The real-time PCR assays revealed, that this strain hyperproduces two different multidrug efflux-pumps, namely the MexAB-OprM and the MexXY-OprM. According to the MLST typing analyses, this strain belongs to a clonal complex, previously identified in VIM metallo- β -lactamase producers in Hungary, namely CC11. Interestingly, the pan-resistant strain was isolated from a polytraumatised Romanian citizen on admission to the hospital of Szeged. This suggests the possibility, that this strain was imported to Hungary from abroad.

P1498 **Emergence and persistence of multidrug-resistant *Pseudomonas aeruginosa* serogroups O11 and O12**

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Objectives: In 1989 Pitt et al. (Epidemiol Infect. Dec;103(3):565–76) reported the emergence of a European multidrug resistant (MDR) serotype O12 clone, and in 1998 Tassios et al. (J Clin Microbiol. Apr;36(4):897–901) reported the emergence of MDR in the ubiquitous and dominant serogroup O11. The objective of the present study was to investigate the emergence, spread and actual status of these MDR serogroups in the light of the global *P. aeruginosa* population structure.

Methods: 328 unrelated *P. aeruginosa* clinical CF (43) and non-CF (142), environmental (63) and animal (55) strains, including 61 serotype O11 and 24 O12 strains, collected between 1882 and 2008 in 69 localities (30 countries, 5 continents) were characterised by fingerprinting (FAFLP), MLST (oprI, oprL and oprD genes), pyoverdine receptor gene typing, prevalence of exoS and exoU genes and serotyping. The prevalence of 23 'Antibiotic Resistance Genes' (ARGs) (15 coding for β -lactamases and 8 for aminoglycoside-modifying enzymes) was determined by PCR. The MIC values for 21 antibiotics were determined using the VITEK 2 Advanced Expert System (AES).

Results: In the minimum spanning tree, based on the combination of the characteristics from the 328 strains, we identified 11 clonal complexes (CCs). Fifty-nine strains (22.4%) were MDR, including 14 O11 (23.7%) and 17 O12 (28.8%) strains. Forty-eight of the 58 detected ARGs were found in MDR O11 and O12 strains. Twenty MDR O12 strains, isolated in 9 countries, some of them separated by thousands of miles, were shown to cluster into a very conserved clone. Only clinical non-CF strains isolated post 1980 clustered into this clone. The MDR serotype O11 strains showed, with the exception of some clonal strains, an overall higher genetic divergence. They belonged to 2 distant CCs, which also included environmental and animal strains, but no CF strains. Most members of the O12 clone harboured the two original (1989) ARGs (PSE-1 and AAC(6')II), while others harboured recent ARGs (e.g. VIM-8).

Conclusion: We suggest that MDR O11 *P. aeruginosa* epidemic strains are members of two widespread and successful CCs that were selected from the environment, in different locations and on several occasions, adapted to the high care niche, and dispersed in hospitals. MDR O12 strains are probably the offspring of a minority clone, which was locally