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Abstract (oral session)

**Development and validation of a multiplex polymerase chain reaction (PCR) assay for identification of the epidemic ST-258 *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* clone**

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**Objectives:** The molecular mechanisms responsible for the epidemiologic success of the ST-258 KPC-producing *K. pneumoniae* (KPC-KP) clone remained unclear. Through comparative genomic study, we have previously identified several unique genes of this clone. Our aims were to 1) test for the presence of these genes in a large, multinational collection of various clones of KPC-KP isolates and to 2) validate a multiplex PCR assay for the identification of the ST-258 clone. **Methods:** This was a multinational, retrospective study of KPC-KP isolates, both of the ST-258 (group A) and non-ST-258 (group B) clones, collected in Columbia, Greece, Israel, Italy and the USA during a 6-year period. KPC-negative ST-258 isolates were also included. Typing was performed using PFGE and MLST. Three ST-258 unique chromosomal genes were selected for testing by a multiplex PCR assay: 1) *pilV* homologue, 2) putative transposase, IS66-family (IS66) and a 3) putative phage-related protein (PRP). **Results:** The following 146 isolates were included: 1) Colombia-A-2, B-1; 2) Greece- A-12, B-8 (6 ST); 3) Israel-A-50, B-17 (13 ST) and 9 KPC-negative ST-258; 4) Italy-A-19 and the USA- A-10, B-18 (16 ST). The *pilV* gene was present in 99/102 of ST-258 isolates, including all of the KPC-negative isolates. It was absent in all 44 non-ST-258 strains, hence having a specificity and sensitivity of 100 and 97%, respectively. The 3 *pilV*-negative strains were all isolated in a Long-Term Care institution in Southern Israel; they were tested positive for the IS66 and PRP genes and had a PFGE pattern indistinguishable from other ST-258 isolates from Israel. The sensitivity values of IS66, PRP and a combination of both genes for detecting KPC-KP ST-258 were 87, 90 and 70%, respectively, and the specificity values were 72, 90 and 93%, respectively. **Conclusion:** This multiplex PCR assay provides a reliable and simple tool for detection of the ST-258 clone among KPC KP strains, although confirmation by other typing method may be advisable for *pilV*-negative, PRP-positive isolates in areas where ST258 is highly prevalent. The *pilV*-homologue is unique to ST-258 among KPC-KP strains and is present in almost all ST-258 isolates from across the world, suggesting a possible role for its epidemiological success.