Evaluation of rapid carbapenemases detection methods on *Klebsiella pneumoniae* isolates from 9 European countries

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Background

Nowadays carbapenemase producing Enterobacteriaceae were detected worldwide. Spread of multidrug resistant bacteria in health care facilities leads to increasing health care costs and treatment failures. Rapid detection of carbapenemase productive strains in clinical settings is mandatory but still challenging and expensive.

The aim of the study was to evaluate different rapid methods of carbapenemases detection.

Picture 1. Identified carbapenemases genes (number of strains) in participating countries

Material/methods

During 01.04.2015 – 30.06.2015 Enterobacteriaceae (n=25 237) clinical strains were screened for carbapenem non-susceptibility (by disk diffusion method, Vitek 2 or Phoenix using EUCAST screening criteria) in 38 institutions from 9 countries: Finland, Estonia, Latvia, Lithuania, Russia (St. Petersburg), Poland, Belarus, Ukraine and Georgia. Among them 171 screening positive *Klebsiella pneumoniae* isolates were selected for further investigation. Identification was confirmed by MALDI-TOF (Bruker, Germany). Carbapenem degradation was detected by MALDI-TOF based method using MBT STAR-Carba Kit prototype and a dedicated software module (both Bruker, Germany), susceptibility (MIC) to Meropenem by agar-gradient diffusion method (Liofilchem, Italy) using EUCAST 2016 guidelines and carbapenemases genes were detected by Luminex in-house panel (includes IMP, VIM, KPC, GIM, OXA48 and NDM genes; Picture 1). The correlation between three methods was investigated.

Results

In total, MBT STAR-Carba assay, MIC testing and multiplex PCR were performed for 171 *K. pneumoniae* isolates. Determined MIC values categorized by EUCAST guidelines led to 51% susceptible isolates (n=88), intermediate – 12% (n=21), and resistant – 37% (n=62). Hydrolysis result corresponding to full enzymatic degradation of Imipenem was detected in 47% of isolates (n=81), non-hydrolyzed result – in 51% (n=87) and ambiguous result – in 2% (n=3). Carbapenemase encoding genes were detected in 46% of the strains (n=76). Among Meropenem-resistant and -intermediate isolates (n=83) 90% isolates (n=75) hydrolyzed Imipenem and 10% (n=8) showed no hydrolysis. Among the hydrolyzing isolates (n=81) different carbapenemase encoding genes were detected in 94% (n=76) and 6% (n=5) showed negative PCR results. Only 2 strains (2.3%) with OXA48 positive genes revealed no hydrolysis in the MBT STAR-Carba assay resulting in 97.7% (n=85) correlation between negative MALDI assay and PCR. This fact could be explained by low activity of OXA enzymes and requiring in some cases a prolonged incubation time.

Table 1. Correlation between MBT STAR-Carba assay results and PCR results

PCR result / MALDI assay	Negative *	KPC gene found	VIM gene found	OXA48 gene found	NDM gene found
Hydrolyzed result (n=81)	5 **	1	1	24	50
Non-hydrolyzed result (n=87)	85	-	-	2 ***	-
Ambiguous result (n=3)	3	-	-	-	-

* Negative PCR result to IMP, VIM, KPC, GIM, OXA48 and NDM encoding genes; ** Isolates MIC ranges to Meropenem are 0.5-32 mg/L; *** Isolates MIC ranges to Meropenem are 0.25 and 32 mg/L

Conclusions

Rapid MALDI-TOF based activity assay and molecular multiplex panel showed strong correlation in detection of common carbapenemases of *K. pneumoniae* strains isolated from Europe.

Kasutatud materjalid

- enilabAMR projekti kollektsioon
- Bruker MBT STAR-Carba Kit (Imipenem) – esitatud ECCMID'l 2017. Kit sisaldab: reagendid ja solvendid, sisekontroll
- MBT Compass STAR-BL software module – installeeritud ITK laboris