Evaluation of a rapid method to detect KPC carbapenemase based on Maldi-TOF spectra analysis

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Anna Rita Centonze[1], Anna Oliani[2] Giuseppe Cornaglia[1-2], Annarita Mazzariol[1-2]

[1] Department of Diagnostic and Public Health, Verona University, Italy

[2] UOC Microbiology and Virology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy

Introduction

The resistance to β-lactams in *Enterobacteriaceae*, with particular reference to carbapenems is actually one of the main international concern in the last two decades. In hospitals outbreaks occur with high rates of mortality by clonally related strains of bacteria.

Clinical microbiology lab needs to quickly identify patients who carry bacterial strains positive for carbapenemases production, so as, to contain their spreading and to address proper pharmacological therapy.

The objective of this study is to standardize the use of Maldi-TOF for quickly identify KPC producers by characteristic peaks related to this carbapenemase activity.

Materials and methods

The study included strains isolated from clinical samples of the Microbiology laboratory of the Azienda Ospedaliera Universitaria di Verona, namely:

218 *Klebsiella pneumoniae* strains: 163 carbapenems resistant and 55 cephalosporins resistant by means of ESβL production or by other resistance mechanisms.

30 *K. pneumoniae*, used as control group, susceptible to all β-lactams

139 *Escherichia coli* strains: 122 ESβL producers, 4 with decreased susceptibility to carbapenems, and 13 carbapenemases producers.

Other strains included in the study were:

17 carbapenem-resistant *Enterobacteriacee* strains, carrying either an NDM or VIM enzyme.

Susceptibility testing was performed for all strains by microdilution test following the EUCAST interpretative guideline (Eucast website).

Carbapenemase and ESβL production were confirmed phenotypically by CarbaNP test and ESβL NDP test, respectively. (Nordmann, 2012).

Carbapenemases genes ($bla_{IMP/VIM/KPC}$, bla_{NDM} and bla_{OXA48}) on *K. pneumoniae* and *E. coli* with decreased susceptibility to carbapenems were detected by multiplex and single PCRs. (Dallenne *et al.* 2010)

Maldi-TOF Vitek MS (Biomerieux™) was used to analyze spectra and identify a peak of 11109 Da (Fig. 1) correlated with KPC enzyme (AF Lau *et al.* 2014).

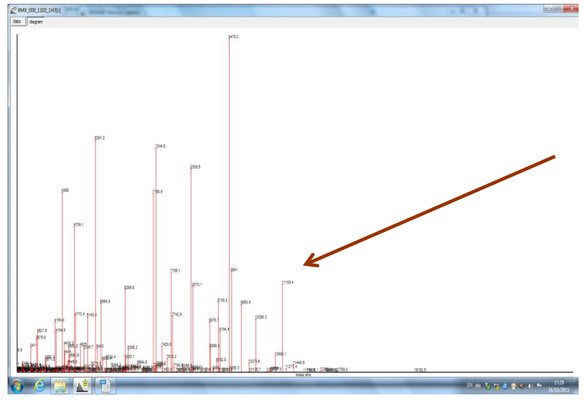
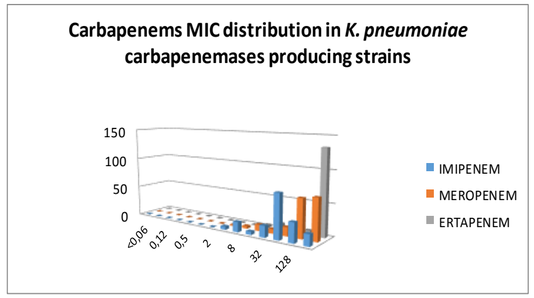


Fig.1 11109Da peak in Maldi-TOF spectra of KPC producing strains

Results

For all strains we confirmed the pattern of resistance by antimicrobial susceptibility testing. The carbapenems MIC distribution in *K. pneumoniae* carbapenemases producing strains is showed in graph. 1.



Graph.1

All carbapenem-resistant strains showed carbapenemase production by a positive CarbaNP test and carried a bla_{KPC} gene. One strain co-carried also a bla_{VIM} gene.

All cephalosporin-resistant strains, showed ESβL production by a positive ESβL NDP test. All showed a negative CarbaNP test, and a negative PCR for all carbapenemase genes.

All *K. pneumoniae* strains (163/163) harboring bla_{KPC} gene, only 0,61% co-carried also a bla_{VIM} gene (1/163)

Discussion and conlcusion

One hundred sixty one out of 163 of *K. pneumoniae* and 12 out 13 *E. coli* carbapenem resistant by Maldi-TOF spectra analysis present the 11109 Da peak (Tab. 1) correlated with the plasmid harboring the bla_{KPC} gene.

	Peak 11109 Da		
	Present	Absent	Total
K. pneumoniae KPC	161	2	163
K. pneumoniae ESβL or Ertapenem R	0	55	55
E.coli KPC	12	1	13
E.coli ESβL o Ertapenem R	0	126	126
K. pneumoniae susceptible	0	30	30
Enterobacteriacee carrying NDM or VIM	0	17	17

Table 1. Results of spectra analysis

Neither the cephalosporin-resistant (ESβL bearing) or the beta-lactams susceptible strains showed the 11109 Da peak in the Maldi-TOF spectra analysis.

Enterobacteriaceae strains harboring carbapenemase other than bla_{KPC}, namely bla_{NDM} and bla_{VIM} did not show the 11109 Da peak in the Maldi-TOF spectra analysis

One hundred seventy three out of 176 *Enterobacteriacee* strains (*K .pneumoniae* and *E .coli*) carbapenem resistant and KPC producers assayed by Maldi-TOF presented the 11109 Da peak correlated with the presence of a KPC enzyme.

The 11109 Da peak was absent in all ESβL producers, susceptible strains and *Enterobacteriaceae* strains harboring different enzyme.

Maldi-TOF is a good and rapid method for identify KPC producers.

Our results confirm that the presence of 11109 Da peak, is a good indicator for the *Enterobacteriaceae* harboring the pKpQIL plasmid coding for KPC enzyme.

http://www.eucast.org/clinical_breakpoints/

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 annarita.mazzariol@univr.it

UNIVERSITÀ di VERONA

Dipartimento di DIAGNOSTICA E SANITÀ PUBBLICA