Detection of carbapenemase-production by MALDI-TOF MS – applicability in a routine laboratory

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BACKGROUND – AIM

The continuously increasing spread of carbapenemase-producing Enterobacteria (CPE) in hospital setting has become a global concern, therefore, the identification of carbapenemase-producing strains should be as fast as possible, since this plays a key role both from therapeutic and from epidemiological points of view.

Several tests are commercially available for the identification of carbapenemase-producing strains. Beyond classical phenotypic and genotypic methods, also MALDI-TOF MS was shown to be suitable for this purpose, as its novel applications enable the direct detection of the enzymatic hydrolytic activity.

*Aim of this study was to investigate the **applicability of MBT STAR-Carba – RUO version (Bruker Daltonik - MALDI-TOF MS carbapenem hydrolysis kit)** to **detect CPE** in a **routine setting with high prevalence of carbapenem-resistant strains**.*

After a comparison of this novel approach with the methods currently used in our routine, it was furthermore applied on a subset of well characterized strains and a subset of challenging strains, to explore its advanced potential.

MATERIALS AND METHODS

MBT STAR-BL hydrolysis assay

MBT STAR-Carba hydrolysis assay (Bruker Daltonik, Germany) detects the carbapenemase activity by an automated investigation of the decreasing of the intact imipenem molecules in its mass spectra due to bacterial hydrolysis, after a short incubation with the strain tested.

The antibiotic mass spectrum was analyzed by an automated software prototype, that calculates the hydrolysis rate and displays results as normalized logRQ score plots. **Fig. 1.**

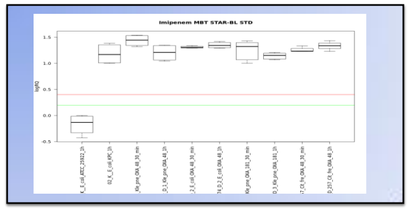


Fig. 1. Normplots displaying results of MBT STAR-BL assay

The cleavage of the imipenem molecules by the bacterial enzyme shifts the logRQ value toward the positive side. . Results below the green line (cut off at logRQ=0.2) are negative (non-hydrolyzing), results above the red line (cut off at logRQ=0.4) are positive (hydrolyzing), while results between the two lines are considered undetermined.

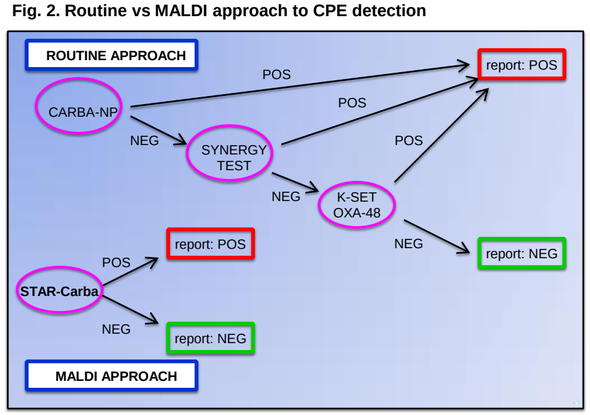
MBT STAR-Carba with strains characterized by PCR

A total of 91 enterobacteria strains with reduced susceptibility to carbapenems, previously characterized by PCR, and 20 highly resistant strains resulted negative with any routine method, were tested with MBT STAR-Carba Kit (RUO version).

Comparison MBT STAR-Carba vs routine

A total of 569 non-duplicated Enterobacteria strains with reduced susceptibility to carbapenems collected from 1st June to 30th September 2016 underwent routine testing for confirmation of carbapenemase production, and were tested in parallel with MBT STAR-Carba assay. **Fig. 2**

Fig. 2. Routine vs MALDI approach to CPE detection



RESULTS

MBT STAR-BL imipenem hydrolysis assay detected **48/49 confirmed CPE** (1 *S. marcescens* KPC+ resulted non-hydrolyzing), and resulted negative for **22/22 ESBL/AmpC-producers,** showing a **sensitivity of 97.9%** and a **specificity of 100%.**

It also detected **9/20 hydrolyzing strains** among the resistant strains **negative to all routine methods. Tab.1**

	STAR-Carba POS	STAR-Carba NEG	tot.
KPC	23	1	24
MBL	20	-	20
OXA-48	5	-	5
tot.	48/49	1/49	49
AmpC	-	10	10
ESBL	-	12	12
tot.	0/22	22/22	22
routine negatives	9	11	20
tot.	9/20	11/20	20

Tab. 1. MBT STAR-Carba results with characterized strains

While the current **routine approach** detected globally **419 CPE, MBT STAR-Carba** hydrolysis assay detected **422 CPE**, and **further 5 strains** that were negative in routine testing. **Tab. 2**.

	ROUTINE+			ROUTINE-	tot.
	CARBA-NP+	DDST+	K-set OXA-48+		
STAR-C.+	266	146	5	5	422
STAR-C.-	1	1	0	144	146
tot.	267	147	5	149	569
	419				

Tab. 2. MBT STAR-Carba with routine strains

The **routine approach** allowed to provide a conclusive result within 3-4 h in **267/569 (46.9%)** strains**, MBT STAR-Carba** in **534/569 (93.8 %).**

DISCUSSION

In this study, **MBT STAR-Carba imipenem hydrolysis assay was shown to be a reliable and robust method to detect carbapenemase-producing strains.**

If applied in routine, a MBT STAR-Carba hydrolysis assay based diagnostic strategy would have enabled to detect, with **one stand-alone test**, the **greatest number of CPE**, of **all classes**, and **reporting time would have been considerably shortened** in a relevant number of cases (267/569).

Moreover, it was able to **detect carbapenemase-producing strains not detected by commonly used methods** in our routine laboratory.

These results suggest that this innovative method **can be applied as "single-step" universal approach to detect CPE**, and it may become a precious tool to prevent a dramatic spread of carbapenem-resistant enterobacteria, when every hour saved counts.

Conflicts of interest

- M. Cordovana, S. Ambretti and M.P. Landini: none
- M. Kostrzewa, K. Sparbier and M. Peer are employees of Bruker Daltonik GmbH)