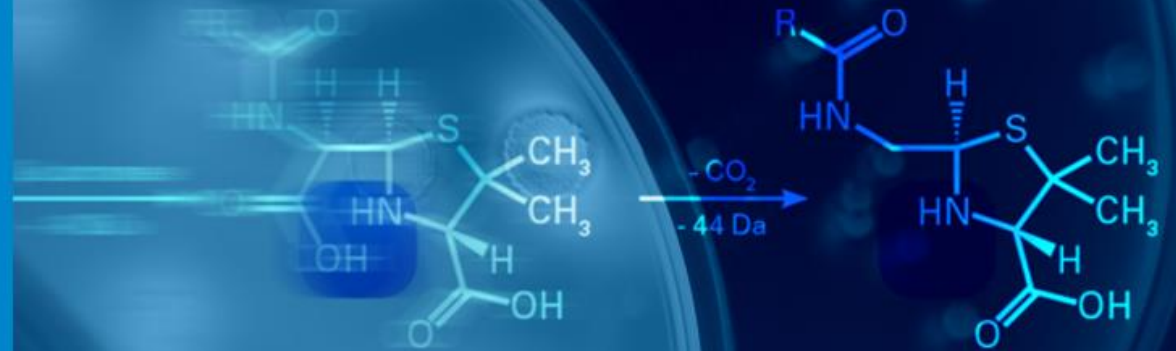
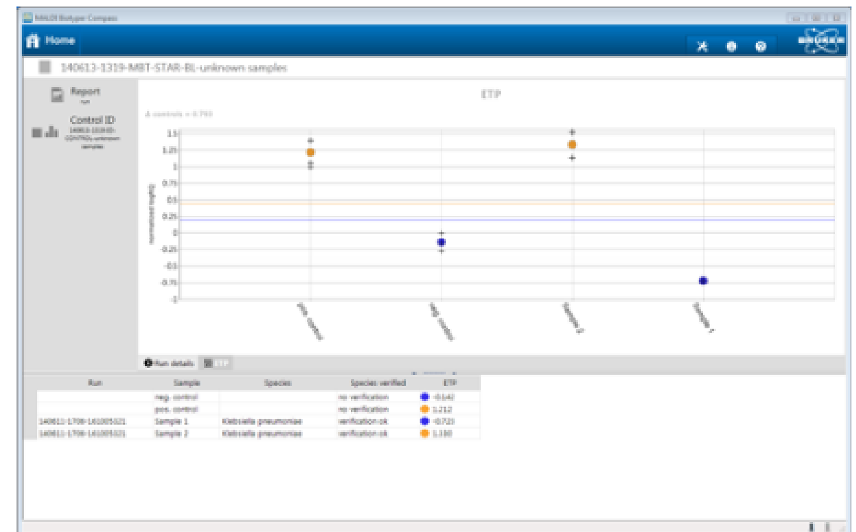
MBT STAR-Carba Kit IVD



Rapid detection of carbapenemase activity via MALDI-TOF MS



The performance of the MBT STAR-Carba IVD Kit was established at three different European sites analyzing a panel of 200 blinded stock strains, each, consisting of the taxonomic units *Enterobacteriacae, Pseudomonas* spp., and *Acinetobacter* spp. according to the standard workflow described in section 7.3. Refer to Table 1 and Table 2 for a detailed overview of the evaluation panel tested.

Prior to the Carba Assay performance, strains were cultured on Columbia blood agar for 18-24 hours at 35 (± 2)°C.

Assay incubation was performed for 30-35 minutes (60-65 minutes for *Acinetobacter* spp.).

Table 1 Taxonomic units used for performance evaluation study per site

Taxonomic unit	Total # of Species	Non-Hydrolyzer (NH)	Hydrolyzer (H)
Enterobacteriaceae	151	74	77
Acinetobacter spp.	26	10	16
Pseudomonas spp.	23	10	13
Total	200	94	106

Table 2 Main carbapenemases genetically characterized and present in the evaluation panel.

Mechanism	OXA	IMP	NDM	VIM	GIM	GES	KPC	Negative	Total
Number	24	16	20	22	3	1	20	94	200

Table 3 Summary of evaluation study results (standard workflow)

Taxonomic unit	Failed	% Accuracy	% Sensitivity	% Specificity
Enterobacteriaceae	13/453	97.1	95.2	99.1
Acinetobacter spp.	1/78	98.7	97.9	100
Pseudomonas spp.	3/69	95.7	94.9	96.7
Total	17/600	97.2	95.6	98.9

Procedural options

For the procedural option of using positively flagged blood culture samples (= **optional workflow**), the performance evaluation was conducted at a single site from a selection of the initial stock isolates (N=100). Positively flagged blood cultures were prepared and analyzed according to the optional workflow described in the IFU with assay incubation performed for generally 60 minutes.

Results of the analysis are summarized in Table 4. All analyses revealed interpretable results.

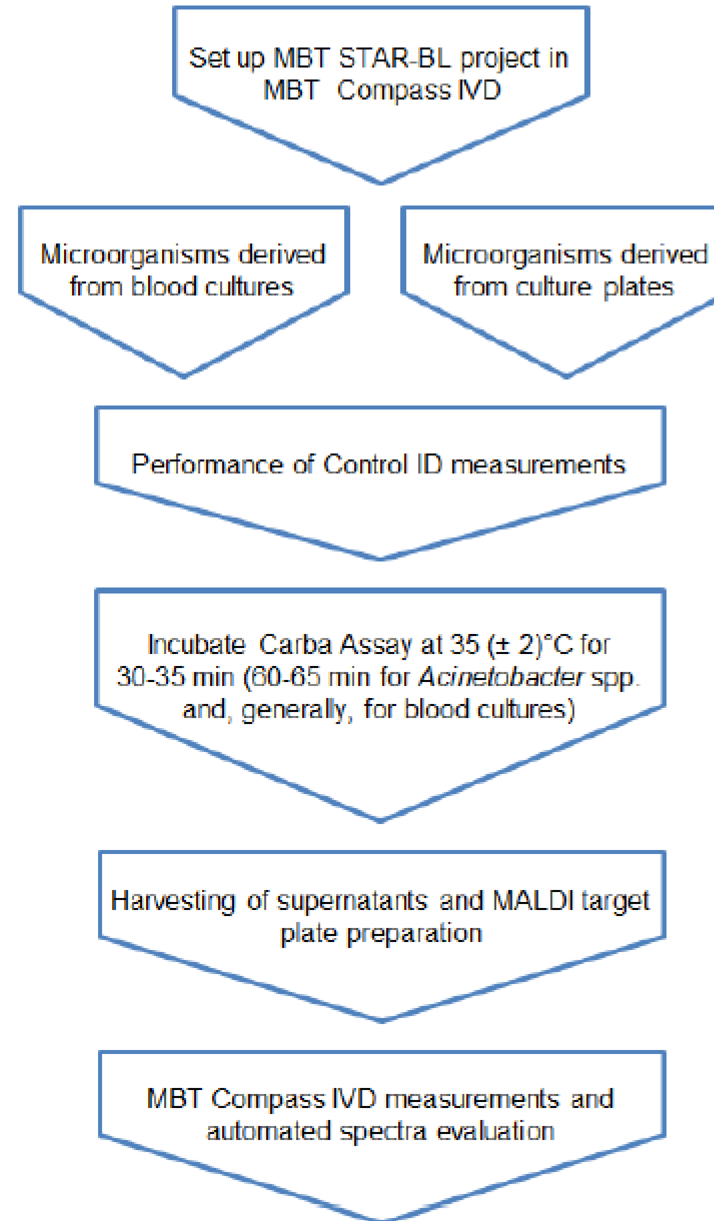
Table 4 Summary of evaluation study results (optional workflow only); 1 site

Taxonomic unit	Failed	% Accuracy	% Sensitivity	% Specificity
Enterobacteriaceae	0/70	94.3	100	88.2
Acinetobacter spp.	6/17	64.7	45.5	100
Pseudomonas spp.	1/13	84.6	90.0	66.7
Total #	7/100	88.0	87.7	88.4

Limitations of the method

- Some GES-type and OXA-type other than OXA-48-type carbapenemase expressing strains might not be detected using the MBT STAR-Carba IVD Kit.
- The Carba Assay can only detect a present carbapenemase activity, requiring an expressed and functional carbapenemase in sufficient quantity and availability. A negative Carba Assay result must be considered potentially "false negative" (non-hydrolyzing) and cannot be used to conclude a diagnostic or therapeutic decision due to the aforementioned reasons or to the possible presence of alternative or additional resistance mechanisms. These mechanisms may include (but are not limited to) modified membrane permeability and porines, efflux pumps, overexpression of β-lactamases (for example, AmpC) and other modifications. Increasing the incubation time may lead to false positive determinations due to autohydrolytic and other effects of the benchmark antibiotic. The final assay results must be assessed by a professional experienced in clinical microbiology.
- Using only the Direct Transfer procedure (DT) for Control ID of the Carba Workflow must be considered as informational only because the DT procedure is not suitable in every case. Please contact your Bruker application specialist for further information.
- Employing the optional workflow performing analyses from positive blood culture derived microorganisms may result in negative hydrolysis results for some *Acinetobacter* spp. due to low cell numbers, low expression of the carbapenemases, or inactivation of carbapenemases during the cell preparation employing the MBT Sepsityper IVD Kit.
- *Acinetobacter* spp. and all species used in the optional Sepsityper workflow require an incubation time of 60-65 minutes.

Summary of the Carba Workflow



6 Required chemicals and materials (not supplied)

For best results, use freshly prepared solutions and chemicals of the highest purity available.

In addition to the MBT STAR-Carba IVD Kit components, the following chemicals and materials are required.

- HPLC-grade water for dissolution of MBT STAR Calibrator (for example, # 22934.K2, VWR International[1])
- IVD Matrix HCCA-portioned (# 8290200, Bruker) (referred to as 'IVD HCCA')
- IVD Bacterial Test Standard (# 8290190, Bruker) (referred to as 'IVD BTS')
- Standard solvent (Acetonitrile 50%, Water 47.5% and Trifluoroacetic acid 2.5%) from Honeywell Riedel-de Haen[2] (# 19182) or SOLUTION OS from VWR International[1] (# PRLS89449.230), which have been tested by Bruker Daltonik GmbH and are recommended for dissolution of IVD HCCA and IVD BTS.
- For processing of positively flagged blood cultures: MBT Sepsityper IVD Kit (# 1834338, Bruker) and respective reagents; for detailed information see Instructions for Use for the MBT Sepsityper IVD Kit.

MALDI target plates

The following MALDI target plates are suitable for Carba Assay and identification determination runs:

- MSP 48 target polished steel BC (# 8281817, Bruker)
- MSP 96 target polished steel BC (# 8280800, Bruker)
- MBT Biotarget 96 (IVD) (# 1839298, Bruker) in combination with MSP Biotarget Adapter (# 8267615, Bruker)

Consumables

- MALDI-TOF compatible pipette tips 0.5–20 µL, 2–200 µL, 50–1000 µL (for example, Eppendorf)
- 1 µL inoculation loops (for example, # L200-1, Simport)

Standard laboratory equipment not provided

- Suitable pipettes for volumes from 1 µL to 1000 µL
- Rack for tubes
- Bench-top microcentrifuge capable of 13,000 to 15,000 rpm (for example, Eppendorf 5424R)
- Vortex mixer
- Temperature control mixer suitable for incubation of assays at 35 (± 2)°C (for example, Eppendorf Thermomixer comfort)
- Other general laboratory equipment

Application of molecular methods for carbapenemases detection

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Due to emerging worldwide spread of carbapenemase-producing Enterobacteriaceae (CPE) rapid detection of carbapenemases is highly important for clinical as well as epidemiological purposes.

AIM

Aim of our study was to evaluate novel phenotypic and genotypic methods for detection of carbapenemases and assessment of their molecular groups dominating in Europe.

MATERIAL AND METHODS

During 01.04.15 – 30.06.15 Enterobacteriaceae (n=25 237) clinical strains were screened for any carbapenem-nonsusceptibility (using EUCAST screening criteria) in 38 institutions of 9 European countries: Finland (FI), Estonia (EE), Latvia (LV), Lithuania (LT), Russia (RU, St. Petersburg), Poland (PL), Belarus (BY), Ukraine (UA) and Georgia (GE).
Totally 259 screening positive (1% of all screened ones) Enterobacteriaceae strains were isolated. These isolates were sent to Estonian reference centers (University of Tartu, SYNLAB Eesti and East Tallinn Central Hospital) for further studies:

- Species ID confirmation by MALDI-TOF MS (Bruker)
- Phenotypic resistance detection
    - Meropenem MIC by agar gradient method (Liofilchem)
    - Resistance profile with Phoenix gram-negative panel NMIC-417 (Becton Dickinson).
- Carbapenemase production confirmation by following assays
    - Imipenem degradation by MALDI-TOF MS based method using MBT STAR-Carba kit (Bruker)
    - Detection of carbapenemases encoding genes by Luminex in-house panel (includes IMP, VIM, KPC, GIM, OXA48, NDM)
    - Whole genome sequencing (WGS) using Illumina and ResFinder 2.1 database for description of carbapenemase and other beta-lactamase genes

RESULTS

Totally **171 carbapenemase screening positive** Enterobacteriaceae strains were confirmed as ***Klebsiella pneumoniae***, 19 as *Escherichia coli* and 69 as other Enterobacteriaceae species. Further we analysed *K. pneumoniae* strains.
87 *K. pneumoniae* strains were carbapenemase negative by all three confirmation methods (not CPEs; 50.9% of all screening positive ones), 74 positive by all methods (confirmed CPEs; 43.3%), 6 positive by two methods (probable CPEs; 3.5%) and 4 positive by only one method (possible CPEs/unclear cases; 2.3%).
Meropenem MIC varied in all groups from sensitive to resistant, however medians in CPEs and probable CPEs groups were higher than in non-CPE group (Table 1).
Out of 80 confirmed and probable CPEs cases Phoenix gave CPE warning in 72 (90%) cases. 5 strains were sensitive to meropenem, 5 to ertapenem, 8 to imipenem and three CPE strains (2 NDM, 1 OXA-48) were sensitive to all 3 determined carbapenems by Phoenix.
In all screening positive strains several ESBL and other beta-lactamase genes were found.

Table 1. Properties of carbapenem screening positive *K. pneumoniae* strains

Number of pos CPE tests	WGS	STAR-Carba	Luminex-Carba	Carbapenemase genes	Number of strains	Country of origin (number of strains)	Meropeneme MIC range (median) mg/L	Other beta-lactamase genes detected (number of strains)
3/3	POS	POS	POS	NDM-1	48	RU (45); BY (2); EE (1)	1.5 - 32 (32)	SHV-188 (48); OXA-1 (46); CTX-M-11 (41); OXA-9 (13); CTX-M-3 (3); TEM-98 (2); CTX-M-124 (1)
	POS	POS	POS	OXA-48	24	RU (13); BY (7); GE (4)	0.75-32 (32)	SHV-188 (23); CTX-M-11 (22); OXA-1 (18); TEM-1A (10); OXA-9 (3); SHV-53 (1)
	POS	POS	POS	KPC-2	1	RU	32	OXA-9; TEM-198; SHV-123
	POS	POS	POS	VIM-5	1	LV	0.75	SHV-188; CTX-M-11;
2/3	NEG	POS	POS	NDM	2	LT (1); RU (1)	12; 32	SHV-188 (2); OXA-1 (2); CTX-M-11 (1); TEM-1A (1)
	POS	POS	NEG	NDM-1	2	RU	6; 32	SHV-188 (2); OXA-1 (2); CTX-M-11 (2); TEM-150 (1); OXA-9 (1)
	POS	NEG	POS	OXA-48	2	RU	0.25; 32	SHV-188 (2); CTX-M-11 (2); OXA-1 (2); TEM-1A (1)
1/3	NEG	POS	NEG	-	3	RU	0.5; 4; 8	SHV-188 (3); CTX-M-11 (2); TEM-1A (1); OXA-9 (1);
	POS	NEG	NEG	NDM-1	1	BY	0.094	SHV-188; CTX-M-11; OXA-1; TEM-1A
0/3	NEG	NEG	NEG	-	87	PL (29); EE (13); LV (13); BY (12); RU (9); LT (8); UA (2); FI (1)	0.032 - 32 (0.094)	SHV-188 (78); CTX-M-11 (63); OXA-1 (53); TEM-1 (53); OXA-9 (27); DHA-1 (18); SHV-112 (3); TEM-150 (2); CTX-M-5 (2); OXA-72 (1); SHV-122 (1); CTX-M-3 (1); CTX-M-14 (1); SHV-187 (1)

34th annual meeting of NSCMID

31 August - 3 September 2017

Tórshavn, Faroe Islands

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	POS	POS	POS	KPC-2	1	RU	32	OXA-9; TEM-198; SHV-123
	POS	POS	POS	VIM-5	1	LV	0.75	SHV-188; CTX-M-11;
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	POS	POS	NEG	NDM-1	2	RU	6; 32	SHV-188 (2); OXA-1 (2); CTX-M-11 (2); TEM-150 (1); OXA-9 (1)
	POS	NEG	POS	OXA-48	2	RU	0.25; 32	SHV-188 (2); CTX-M-11 (2); OXA-1 (2); TEM-1A (1)
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CONCLUSIONS

- Half of screening positive *K. pneumoniae* strains were negative for carbapenemases production/encoding genes
- All CPE confirmation assays missed some few (2.5%) carbapenemases cases in *K. pneumoniae* strains
- Sensitivity to several carbapenems by different assays varied in carbapenemase producing *K. pneumoniae* strains
- Thus phenotypic sensitivity test and automatic warning system may fail to detect some CPE cases
- NDM-1 and OXA-48 were dominating carbapenamase genes most commonly found in St Petersburg, Russia

Direct-On-Target Microdroplet Growth Assay for Rapid Detection of Carbapenem Resistance in Pseudomonas aeruginosa using MALDI-TOF Mass Spectrometry

FULL TEXT

Article *in* Open Forum Infectious Diseases 4(suppl_1):S598-S598 · October 2017
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