

Next Generation Molecular Diagnostics: Microarrays, MALDI-TOF and PCR/ESI-MS

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Appreciation and Disclosures

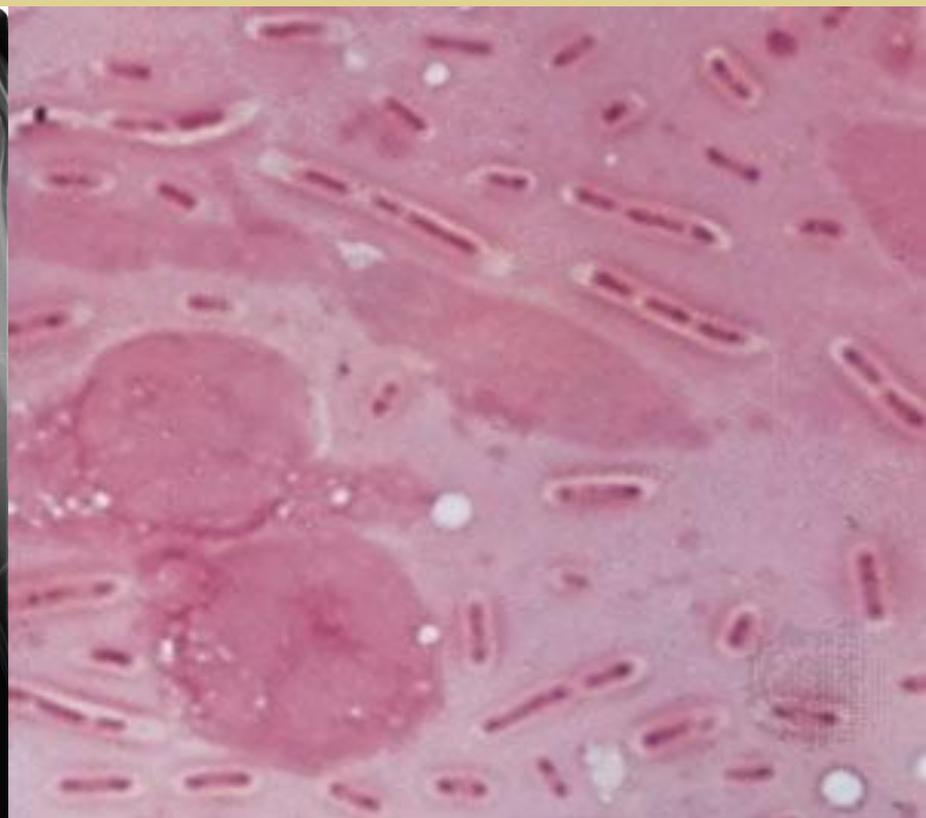
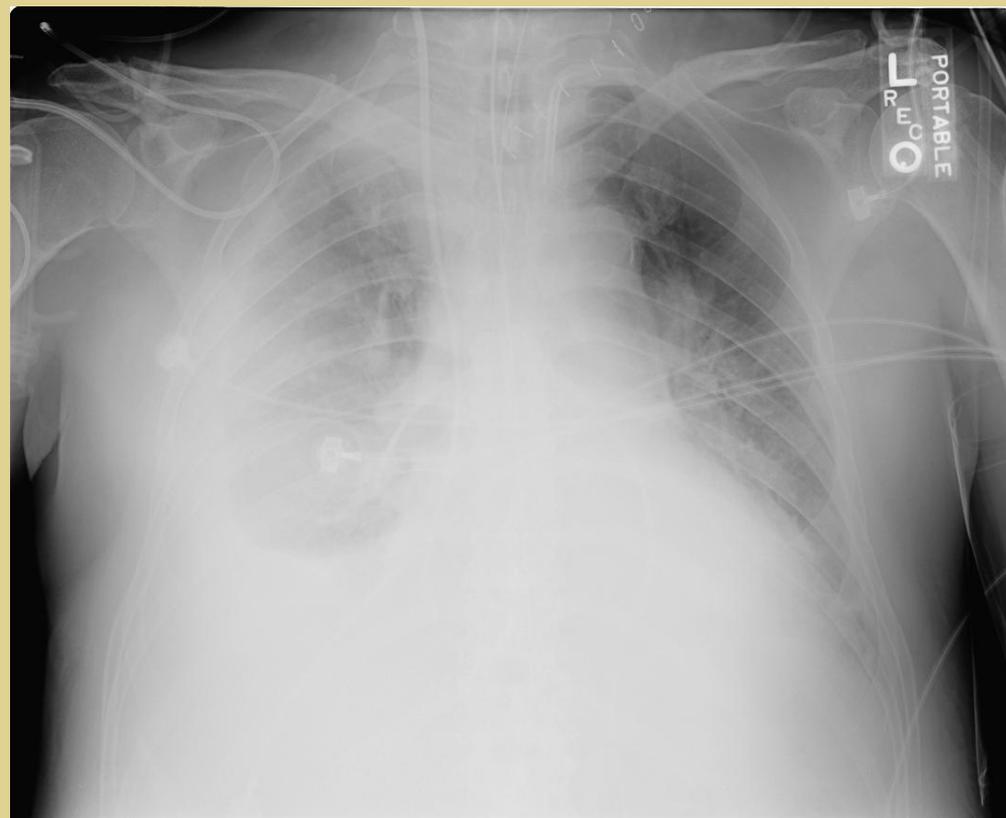
- The organizing committee, Dr. Keith Kaye
- Support from VA and NIH

Apology

- “The view of a clinician in an academic medical practice in charge of a training program that needs to be cost conscious”

Case history

- **63 yo man with esophageal cancer, s/p resection, develops post op fever.**
- **Symptoms = chills and malaise.**
- **PE = rales right lower lung**
- **CBC, WBC= 14K, O2 saturation=80; intubated**
- **CXR++**
- **Sputum exam= GNRS**



**Microbiological diagnosis?
Took 4 days!!**



KPC *K. pneumoniae*

AMIKACIN	R
AMPICILLIN	R
CEFAZOLIN	R
CEFTAZIDIME	R
CIPROFLOXACIN	R
TRIMETH/SULFA	R

<u>IMI/MERO-PENEM</u>	4 ug/ml → (> 64)
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GENTAMICIN	S
AMPICILLIN/SUL	R
CEFOTETAN	R
CEFEPIME	R
PIP/TAZO	R

The battle between pathogens and host
***Find the enemy before they
find you***



“Main Challenges: ESBLs, AmpCs, and Carbapenemases”

- Class A ESBLs (TEM-26, SHV-2, -5, -12)
- CMY-2, DHA, AmpC
- KPCs
- MBLs (NDM, VIM, IMP, SPM)
- OXA-48
- OXAs (-23, -24) in *Acinetobacter* spp.

“(Other) Main Challenges: MRSA, VRE, PRPn, ER GAbHS, PRGC, efflux”

- *mecA*
- *vanA*
- Altered *pbp* genes
- *adeRS, adeABC*
- *mexT*
- *ponA,*
- *armA*
- *gyrA*



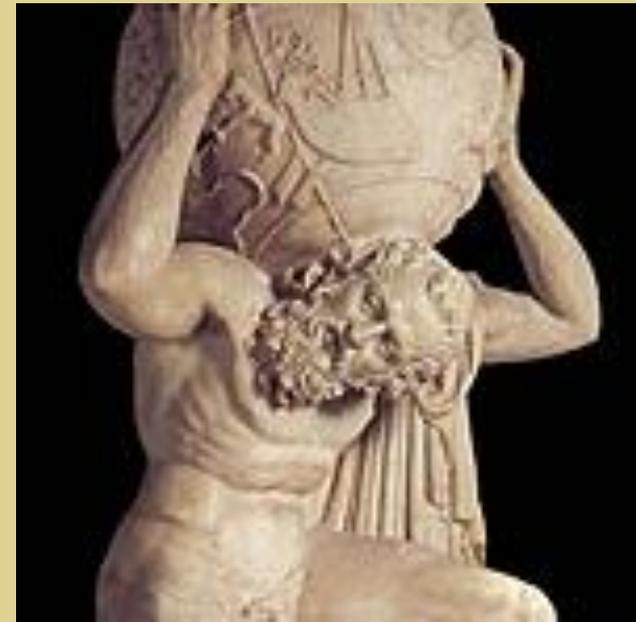
and the beat goes on.....

Criteria for Future diagnostic strategies

- Have to do better than the clinician
- Added value: have to provide more than interpretation of the Gram stain
- Economical
- Accurate
- Easy

Example of molecular technologies that will likely make it to clinical trials very soon

- **Nucleic acid microarrays**
- **MALDI-TOF**
- **PCR/ESI-MS**

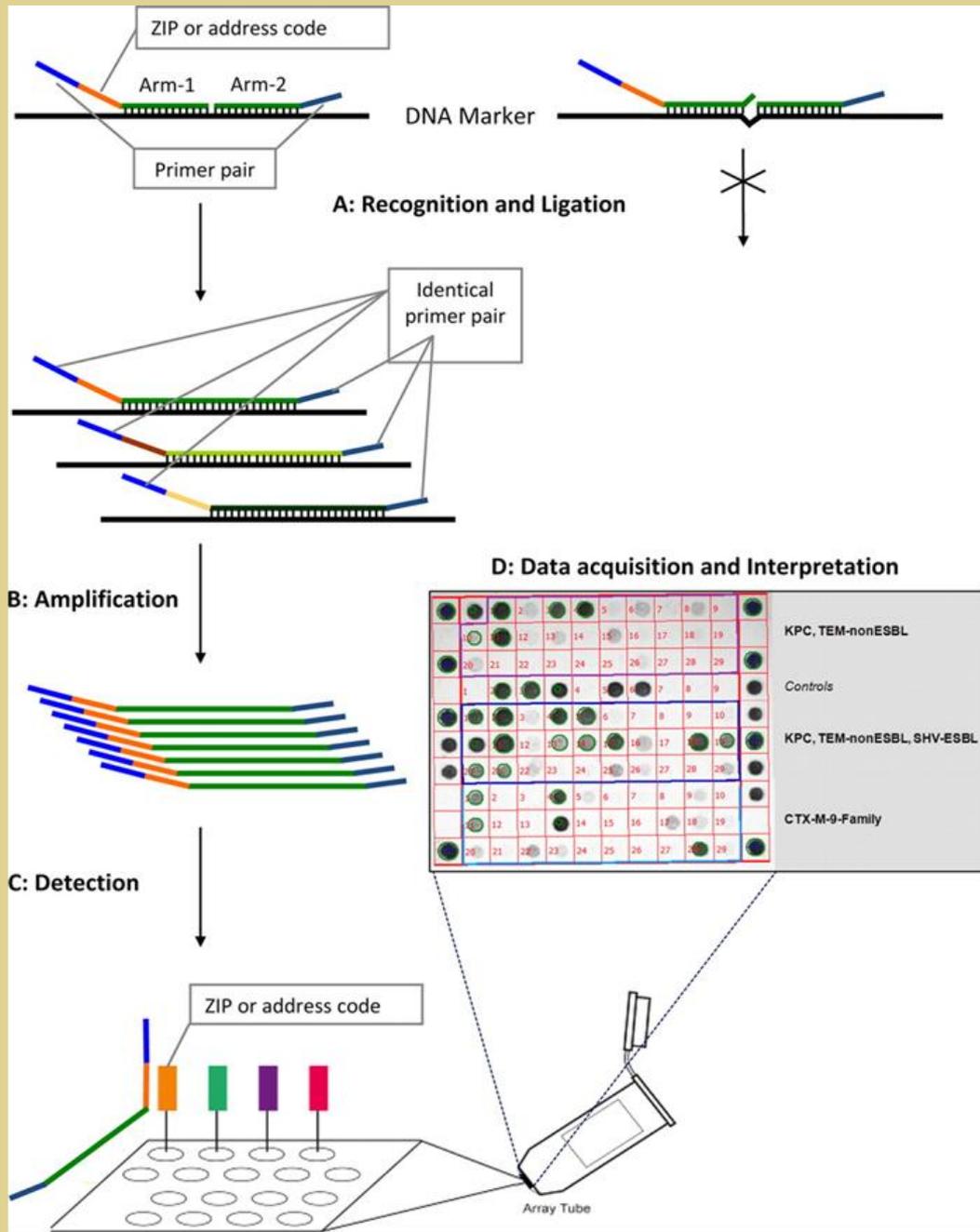


Nucleic acid microarrays: Check Points™

Enterobacteriaceae
P. aeruginosa
A. baumannii



Rapid diagnostics and molecular epidemiology
RT PCR



Microarray technologies possess a high “multiplexing capacity” and can be used for detecting different bla

Evaluation of a Commercial Microarray System for Detection of SHV-, TEM-, CTX-M-, and KPC-Type β -Lactamase Genes in Gram-Negative Isolates^{∇†}

Andrea Endimiani,^{1,2} Andrea M. Hujer,^{1,2} Kristine M. Hujer,^{1,2} Julian A. Gatta,¹
Andrew C. Schriver,¹ Michael R. Jacobs,³ Louis B. Rice,¹ and Robert A. Bonomo^{1,2,4,5*}

A total of 106 Gram-negative strains were tested.

- 1. S/S for *bla*SHV, 98.8% and 100%;**
- 2. S/S for *bla*TEM, 100% and 96.4%;**
- 3. S/S *bla*CTX-M and *bla*KPC, 100% and 100%.**

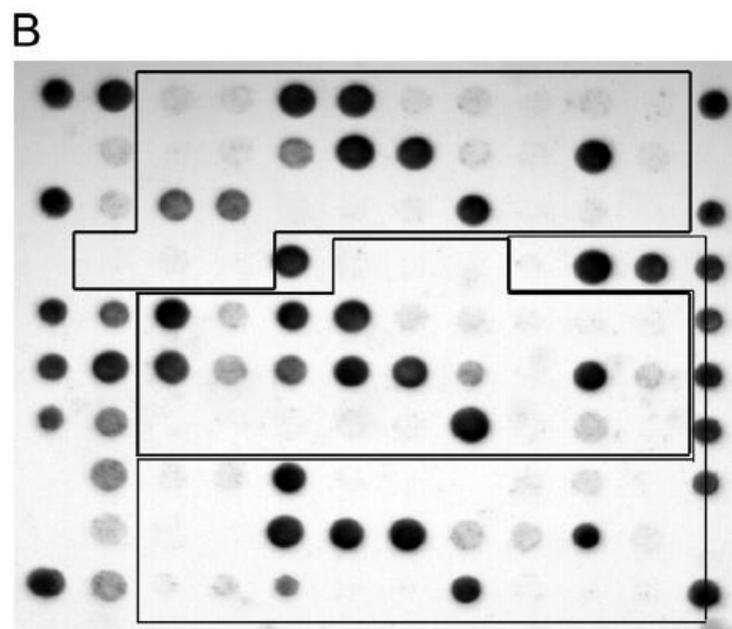
DIAGNOSTICS

Evaluation of a DNA Microarray (Check-MDR CT102) for Rapid Detection of TEM, SHV, and CTX-M Extended-Spectrum β -Lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 Carbapenemases[∇]

Thierry Naas,^{1*} Gaëlle Cuzon,¹ Pierre Bogaerts,² Youri Glupczynski,² and Patrice Nordmann¹

A

I _{Hyb} C	NDM E104K	TEM G238S	DNAC	TEM all	TEM R164S	TEM R164C	TEM R164H	IMP 240E	IMP E240K
CTR	NDM	TEM G238S	DNAC	SHV	SHV 238G	SHV G238S	SHV G238A	SHV 240E	SHV E240K
CTR	OXA48	OXA48	negC	VIM	VIM	CTX- M1	CTX- M2	CTX- M9	CTX- M8-23
VIM all	KPC	KPC	I _{Hyb} C	VIM all	KPC	KPC	VIM all	KPC	KPC
CTR	NDM E104K	TEM G238S	DNAC	TEM all	TEM R164S	TEM R164C	TEM R164H	IMP	IMP
I _{Hyb} C	NDM	TEM G238S	DNAC	SHV	SHV 238G	SHV G238S	SHV G238A	SHV 240E	SHV E240K
CTR	OXA48	OXA48	negC	VIM	VIM	CTX- M1	CTX- M2	CTX- M9	CTX- M8-23
CTR	NDM E104K	TEM G238S	DNAC	TEM all	TEM R164S	TEM R164C	TEM R164H	IMP	IMP
negC	NDM	TEM G238S	HybC	SHV	SHV 238G	SHV G238S	SHV G238A	SHV 240E	SHV E240K
CTR	OXA48	OXA48	DNAC	VIM	VIM	CTX- M1	CTX- M2	CTX- M9	CTX- M8-23



144 Gram-negative strains

The sensitivity and specificity were 100%

Multicenter Evaluation of a New DNA Microarray for Rapid Detection of Clinically Relevant *bla* Genes from β -Lactam-Resistant Gram-Negative Bacteria[∇]

Pierre Bogaerts,^{1*} Andrea M. Hujer,^{2,3} Thierry Naas,⁴ Roberta Rezende de Castro,¹
Andrea Endimiani,^{2,3} Patrice Nordmann,⁴ Youri Glupczynski,¹
and Robert A. Bonomo^{2,3,5,6}

4460 BOGAERTS ET AL.

ANTIMICROB. AGENTS CHEMOTHE

TABLE 2. DNA array results for 25 isolates harboring chromosomal AmpC-encoding genes

Species carrying plasmid-encoded AmpC progenitor (AmpC type)	No. of isolates tested	No. of isolates with array detection of gene group						% of progenitor detected by array
		<i>bla</i> _{CMY-2}	<i>bla</i> _{DHA}	<i>bla</i> _{FOX}	<i>bla</i> _{ACC}	<i>bla</i> _{ACT/MIR}	<i>bla</i> _{CMY-1}	
<i>Citrobacter freundii</i> (CMY-2)	10	4	0	0	0	0	0	40
<i>Enterobacter cloacae</i> (MIR) + <i>E. coli</i> P99	6	0	0	0	0	4	0	67
<i>Enterobacter asburiae</i> (ACT)	1	0	0	0	0	1	0	100
<i>Morganella morganii</i> (DHA)	4	0	4	0	0	0	0	100
<i>Aeromonas hydrophila</i> (CMY-1)	2	0	0	0	0	0	0	0
<i>Hafnia alvei</i> (ACC)	2	0	0	0	1	0	0	50

100 S/S for plasmid AmpCs

Increasing prevalence and dissemination of NDM-1 metallo- β -lactamase in India: data from the SMART study (2009)

Christine Lascols^{1*}, Meredith Hackel¹, Steven H. Marshall², Andrea M. Hujer³, Sam Bouchillon¹, Robert Badal¹, Daryl Hoban¹ and Robert A. Bonomo^{2–5}

To investigate the β -lactamase background of ertapenem non-susceptible isolates (235) for the presence of the most commonly detected carbapenemase genes, *blaKPC*, *blaOXA-48* and *blaVIM*, and the newly described *blaNDM-1*.

MOLECULAR EPIDEMIOLOGY

SMART STUDY

- 66 isolates (28%) had a carbapenemase gene,
 - *bla*NDM-1 in 33
 - *bla*NDM-1 and *bla*OXA-48 in 2;
- *bla*KPC (n = 23), *bla*VIM (n = 7) and *bla*OXA-48 (n = 3).
- All *bla*NDM-1-carrying isolates were from patients in India and comprised five different species.

Using Nucleic Acid Microarrays To Perform Molecular Epidemiology and Detect Novel β -Lactamases: a Snapshot of Extended-Spectrum β -Lactamases throughout the World

Christine Lascols,^a Meredith Hackel,^a Andrea M. Hujer,^d Steven H. Marshall,^c Sam K. Bouchillon,^a Daryl J. Hoban,^a Stephen P. Hawser,^b Robert E. Badal,^a and Robert A. Bonomo^{c,d,e,f}

J Clin Microbiol. 2012 Feb 8



Worldwide distribution of the 1,093 clinical isolates tested.

Results

- 1000+ isolates studied
- “Molecular snapshot” of *bla*ESBL genes in a current global population,
- Determine that *bla*CTX-M-15 is still the dominant ESBL, and provide the first report of new β -lactamase variants,
 - *bla*SHV-129 and *bla*KPC-11;
- 1/500 new *bla* genes

Ex: Survey of “resistance genes” in *A. baumannii*

<u>bla</u>	<u>AMEs</u>	<u>QRDR</u>	<u>RND</u> <u>Efflux pumps</u>	<u>OMPs</u>	<u>Tet</u>
ADC	<i>aacC1</i>	<i>gyrA</i>	AdeABC	HMP-AB	<i>tetA</i>
OXA	<i>aacC2</i>	<i>parC</i>	AdeM	OmpA	<i>tetB</i>
IMP	<i>aacC3</i>		AdeIJK	33-36 kDa	<i>tetM</i>
VIM, GIM	<i>aacA4</i>		AdeS	25/29 kDa	<i>tetX</i>
SIM, SPM, NDM			CraS	CarO	
PER	<i>aphA1</i>		AdeDE		
			<u>Res Is??</u>	OprD (43kDA)	<u>PBPs</u>
TEM*	<i>aphA6</i>		AbaR 1-10	OmpW	
SHV	<i>aadA1</i>		<u>Col^R</u> pmrAB	44, 47kDa, 22	integrans
CTX-M	<i>rmt*</i>				OMVs

Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry - Bruker Biotyper



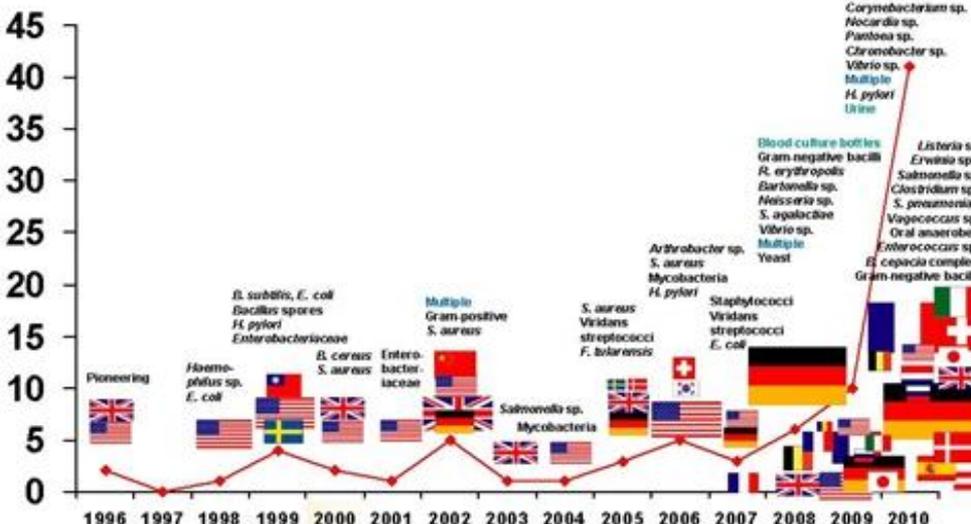
Put plate into instrument and analyze



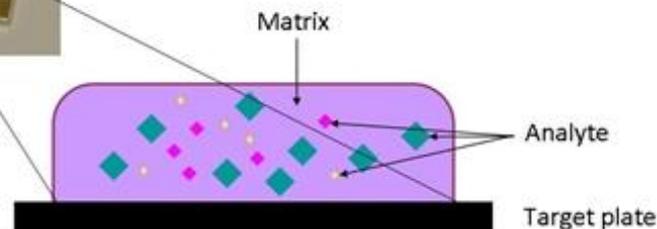
Mass Spectrometry

Bacterial Identification by MALDI TOF Mass Spectrometry¹

Number of papers



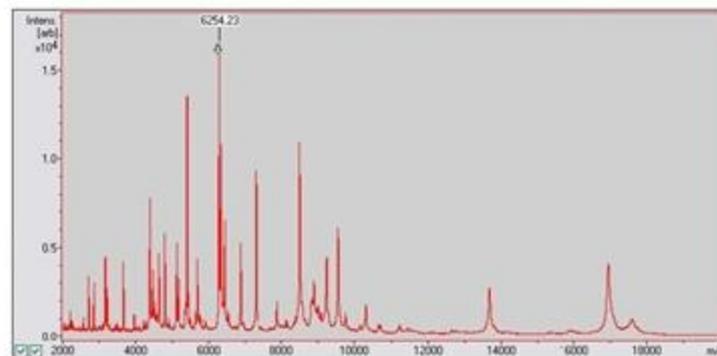
Matrix-Assisted Laser Desorption Ionization



Mass Spectrometry

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Mass Spectrum Generated Compared with Library (Database)



Mass Spectrometry

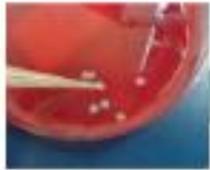
Placed on a steel surface with matrix material

Short laser pulses

Cinnamic acid co-crystallizes the material

Ionization of ribosomal proteins

10^4-10^6 cfu



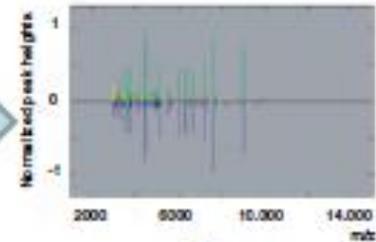
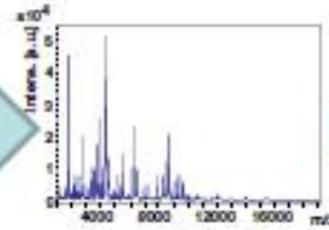
A

B

C

300 samples

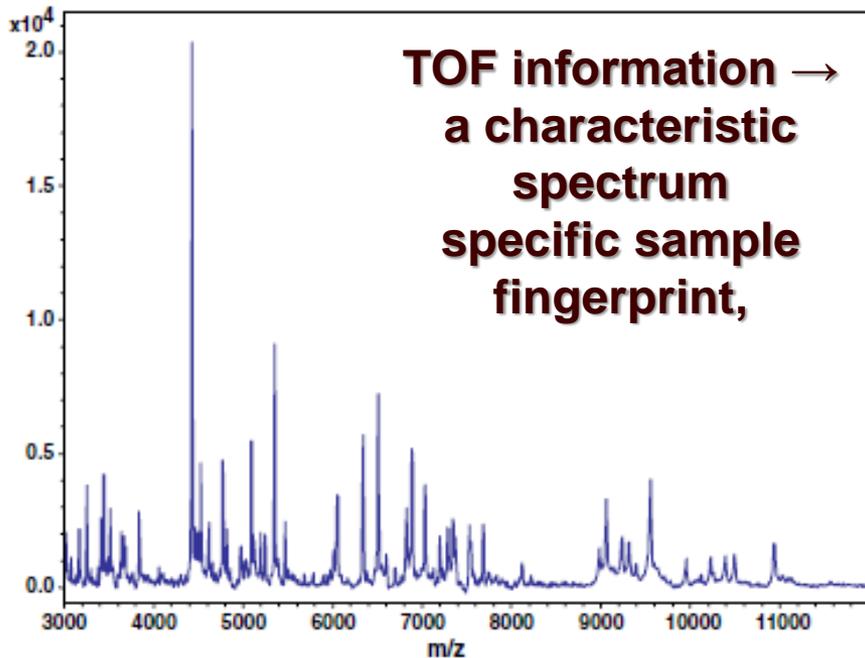
Ionization and mass of proteins



D

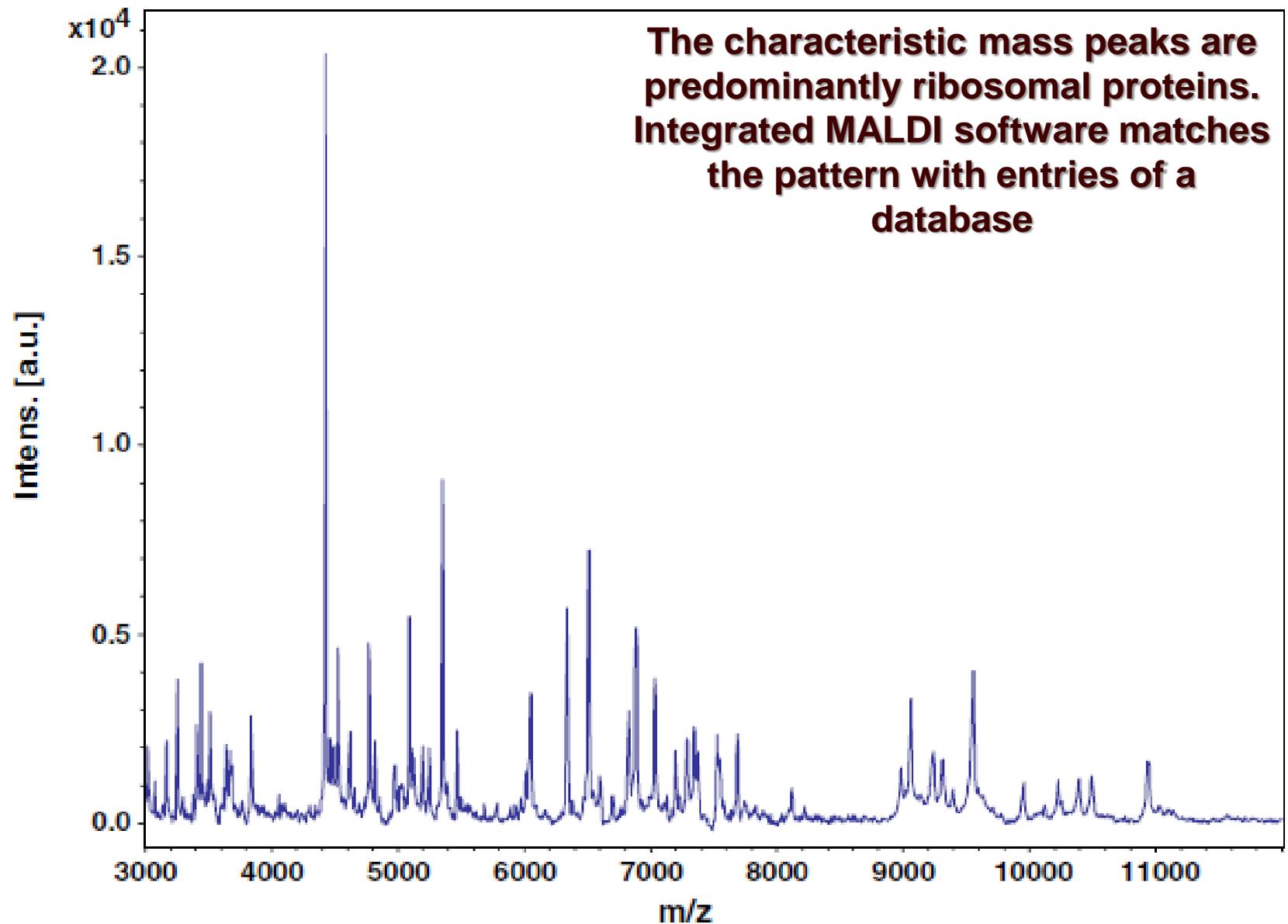
E

TOF information →
a characteristic
spectrum
specific sample
fingerprint,



Ranking	Species Identification	Score Value	NCBI Code
1 (++)	<i>Enterococcus faecium</i> DSM 17050 DSM	2.298	1352
2 (++)	<i>Enterococcus faecium</i> 20218_1 CHB	2.297	1352
3 (++)	<i>Enterococcus faecium</i> 11037 CHB	2.206	1352
4 (++)	<i>Enterococcus faecium</i> DSM 13589 DSM	2.116	1352
5 (++)	<i>Enterococcus faecium</i> DSM 2146 DSM	2.093	1352
6 (++)	<i>Enterococcus faecium</i> DSM 2918 DSM	2.008	1352
7 (+)	<i>Enterococcus faecium</i> PX_21086109_III MLD	1.949	1352
8 (+)	<i>Enterococcus faecium</i> DSM 6177 DSM	1.862	1352
9 (+)	<i>Enterococcus faecium</i> VRE_PX_16086218 MLD	1.83	1352
10 (+)	<i>Enterococcus mundtii</i> DSM 4840 DSM	1.739	53346

>2.0 is a valid ID



Challenges

- 16 s-rDNA sequence database comparisons.
- *Shigella* spp. and *E. coli* or *S. pneumoniae* (pneumococcus) and members of the *S. oralis/mitis* group, cannot be distinguished by MALDI-TOF MS.

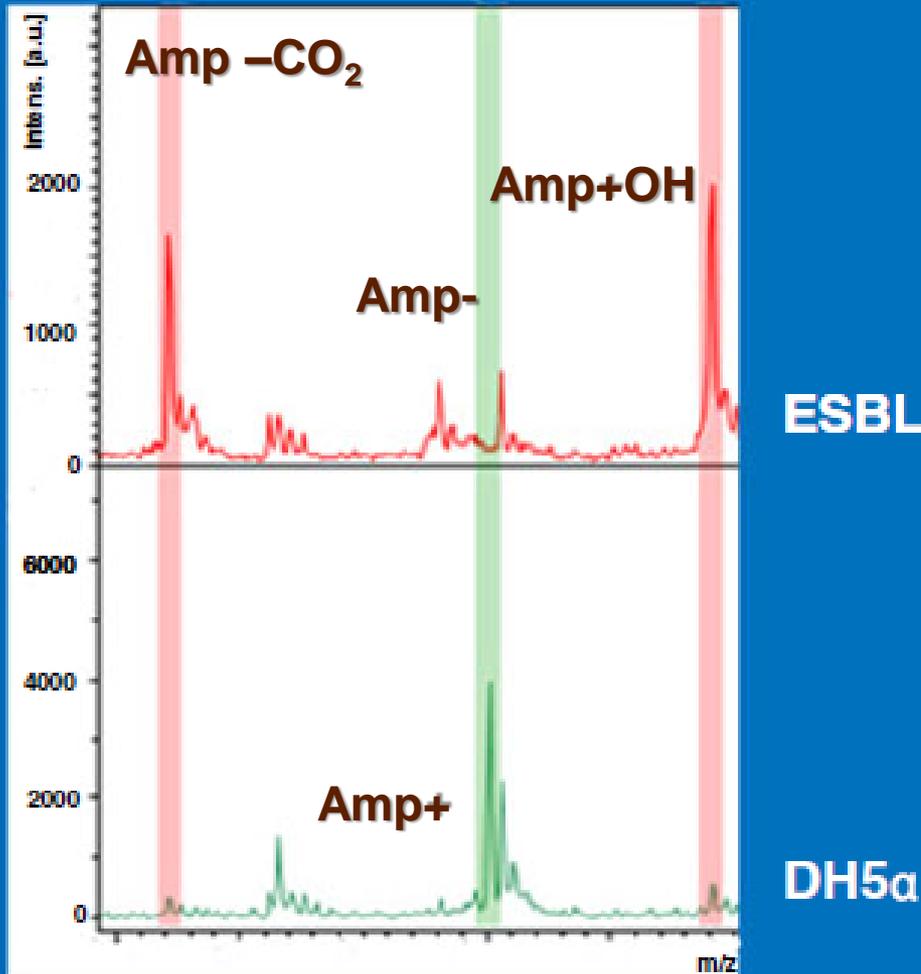
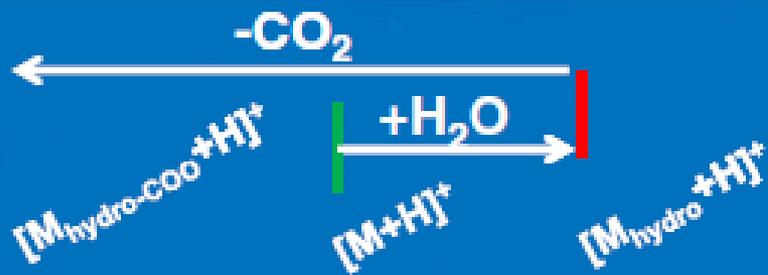
**Finding the pathogen is
not enough?**

**Can one detect
resistance mechanisms?**

Progress so far.....

Application to

β -lactamases



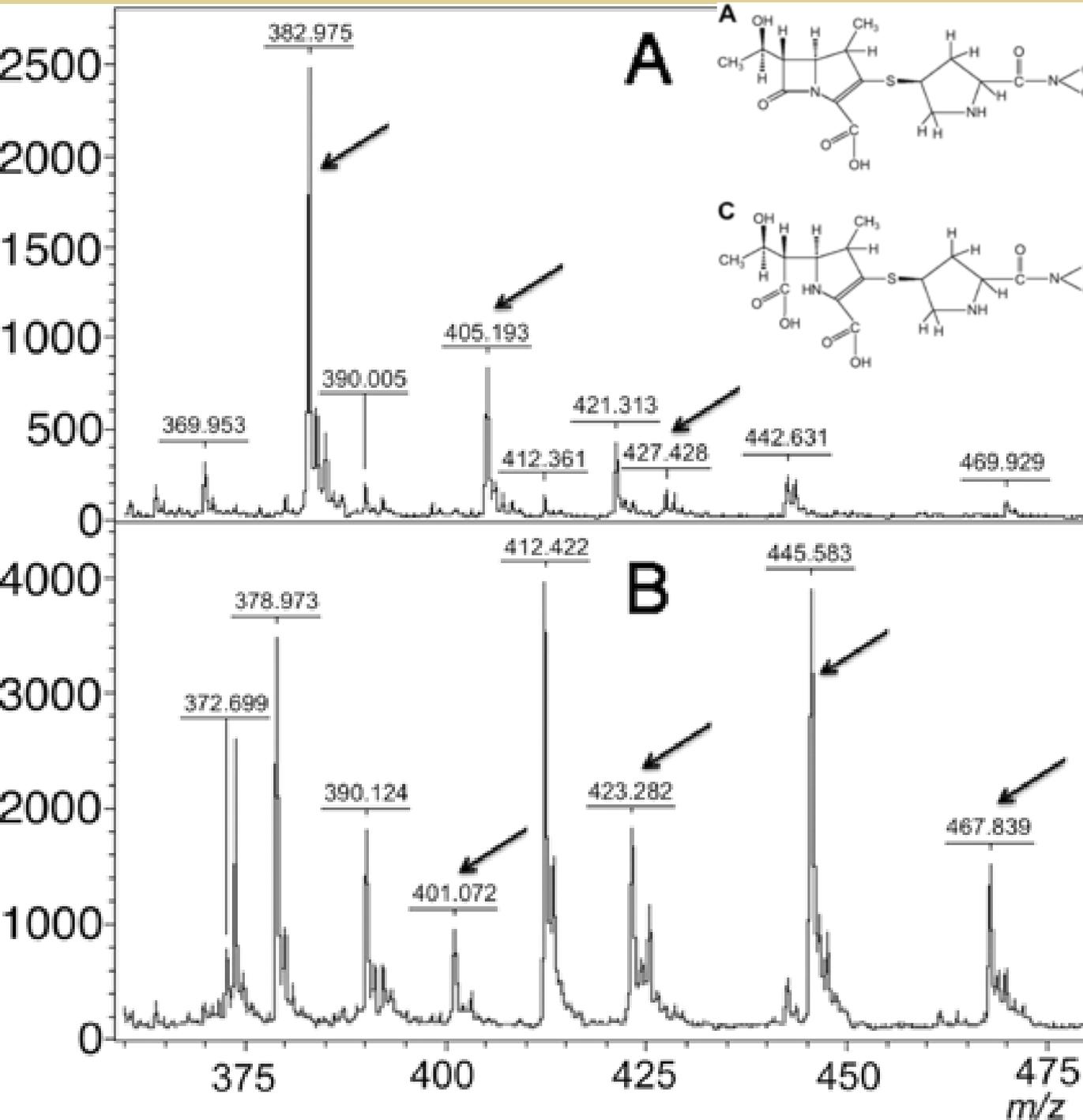
C A B

β -lactamase in *E. coli* p incubation with Amp for 2.5 h.

Amp is detectable only in the supernatant of the β -lactamase (-) control strain DH5a (a).

The supernatant of the ESBL-+ *E. coli* strain reveals the Amp (b) with a mass of + 18 Da according to the addition of H₂O.

Δ -CO₂ product is visible as a 44- Da lower mass peak (c).



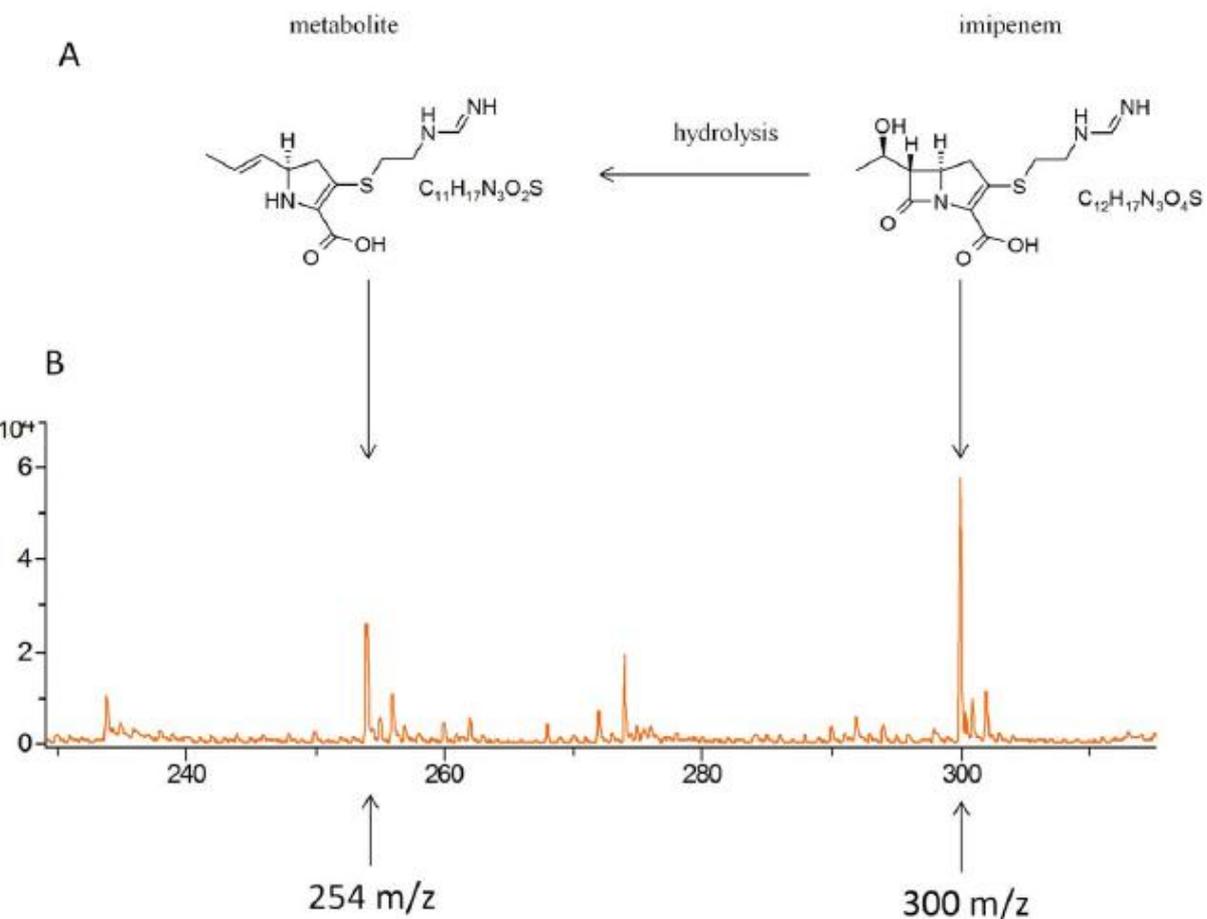
Meropenem, M, 383.464 Da (A), its disodium salt, 427.422 Da (B) MNa₂, a meropenem degradation product with a disrupted amide bond, 401.483 Da (C), and its trisodium salt MNa₃, 467.420 Da (D).

KPC-2 strain

Rapid Detection of Carbapenem Resistance in *Acinetobacter baumannii* Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

Marie Kempf^{1,3}, Sofiane Bakour^{1,2,3}, Christophe Flaudrops¹, Meryem Berrazeg¹, Jean-Michel Brunel¹, Mourad Drissi³, Esma Mesli³, Abdelaziz Touati², Jean-Marc Rolain^{1*}

¹ Aix-Marseille-Université, Unité de Recherche sur les Maladies Infectieuses et Tropicales Émergentes (URMITE), CNRS-IRD-INSEERM UMR 6236, Méditerranée Infection, Faculté de Médecine et de Pharmacie, Marseille, France, ² Département de Microbiologie, FSNV, Université A/MIRA de Béjaïa, Béjaïa, Algeria, ³ Laboratoire Antibiotiques, Antifongiques, Physico-chimie, Synthèse et Activité Biologique (LAPSAB), Université Abou Bekr Belkaïd-Tlemcen, Tlemcen, Algeria

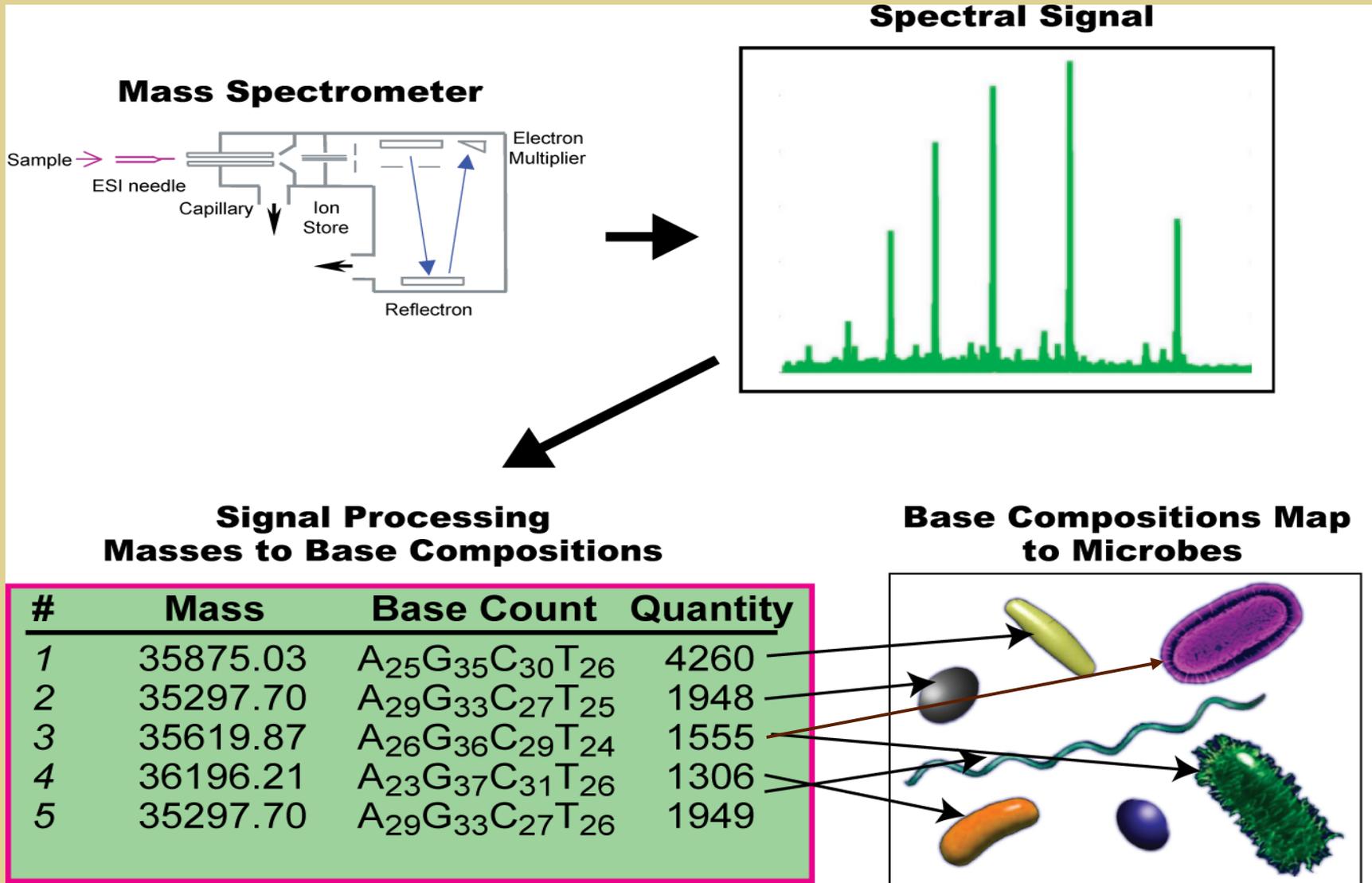


Note, detection of carbapenem resistance

Threat pathogen

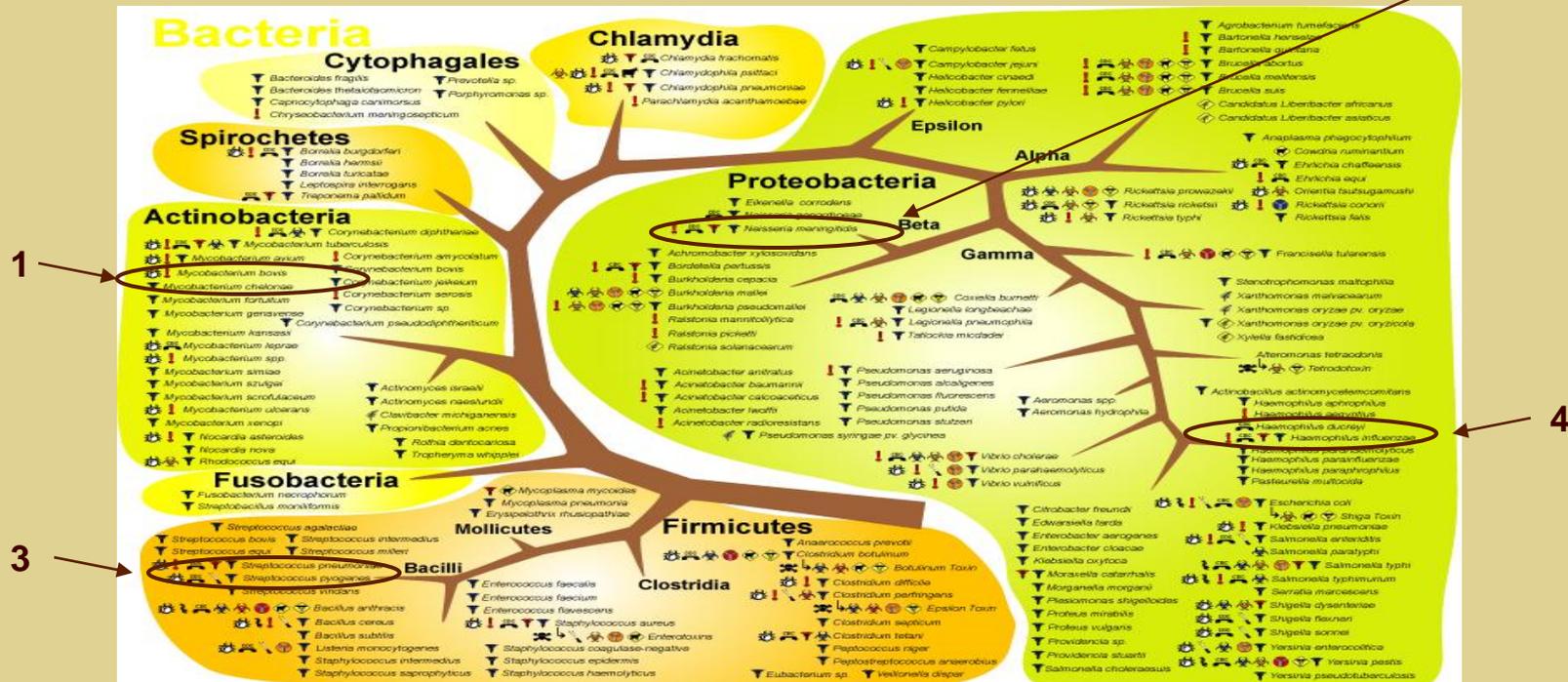
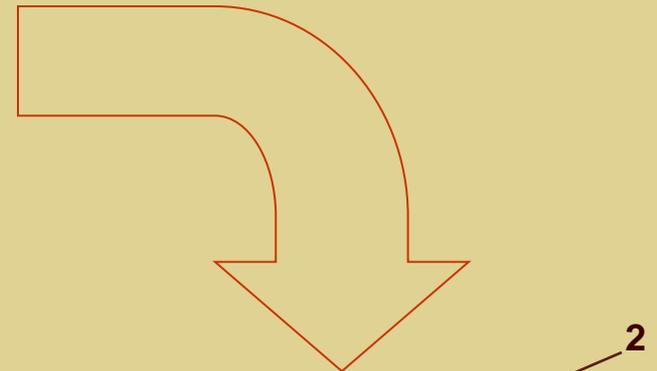


MS Analysis and Signal Processing



Triangulation Using Multiple Primer Pairs

#	Mass	Base Count	Quantity	Quality
1	35875.03	A ₂₅ G ₃₅ C ₃₀ T ₂₆	4260	227
2	35297.70	A ₂₉ G ₃₃ C ₂₇ T ₂₅	1948	966
3	35619.87	A ₂₆ G ₃₆ C ₂₉ T ₂₄	1555	27
4	36196.21	A ₂₃ G ₃₇ C ₃₁ T ₂₆	1306	36
Cal	33856.09	A ₂₀ G ₃₄ C ₂₀ T ₂₂	887	48
Primer Pair 1				
Cal	43515.168	A ₂₅ G ₃₂ C ₂₇ T ₂₉	903	37
Primer Pair 2				
Cal	39079.45	A ₂₆ G ₃₀ C ₂₈ T ₃₂	1093	37
Primer Pair 3				



Progress so far...

Pathogen identification

mec, gyr, par, erm

Application to

β -lactamases

Research letters

Clonal typing
gyrA, *parC*
mecA, PVL, TSST,
mupA, *nucA*

J Antimicrob Chemother 2010
doi:10.1093/jac/dkq207
Advance publication 10 June 2010

C. diff, PSDA, *Candida*,
etc.

Rapid identification of *bla*_{KPC}-possessing Enterobacteriaceae by PCR/electrospray ionization-mass spectrometry

Andrea Endimiani^{1,2}, Kristine M. Hujer^{1,2},
Andrea M. Hujer^{1,2}, Rangarajan Sampath³,
David J. Ecker³ and Robert A. Bonomo^{1,2,4,5*}

RESULTS

- All 74 *bla*_{KPC}-possessing strains; false-positive results were not observed (sensitivity and specificity of 100%).
- *bla*_{KPC-2}⁻ and *bla*_{KPC-3}⁻-possessing strains were correctly reported.
- Not able to discriminate between *bla*_{KPC-6}⁻, *bla*_{KPC-7}⁻ and *bla*_{KPC-8}⁻-containing isolates [these strains were reported as KPC-2 positive (for *bla*_{KPC-6}) or KPC-3 positive (for *bla*_{KPC-7} and *bla*_{KPC-8})].
- Finally, all tested strains were correctly identified at the species level.

Advantages of nucleic acid microarrays

**1. Characterize resistance
determinants**

**2. Perform molecular
epidemiology**

3. Expandable and

**“contemporary” platform: *Check-
MDR Carba*: Real Time detection**

Advantages

MALDI-TOF

- **\$\$\$ (200K), fast—3-4h**
- **Pathogen 97% (depends on library)**
- **Bug on the plate and in the blood-need protein**
- **Resistance determinant detected by chemical assay**
- **New variants ??**
- **No clonal analysis**

PCR/ESI-MS

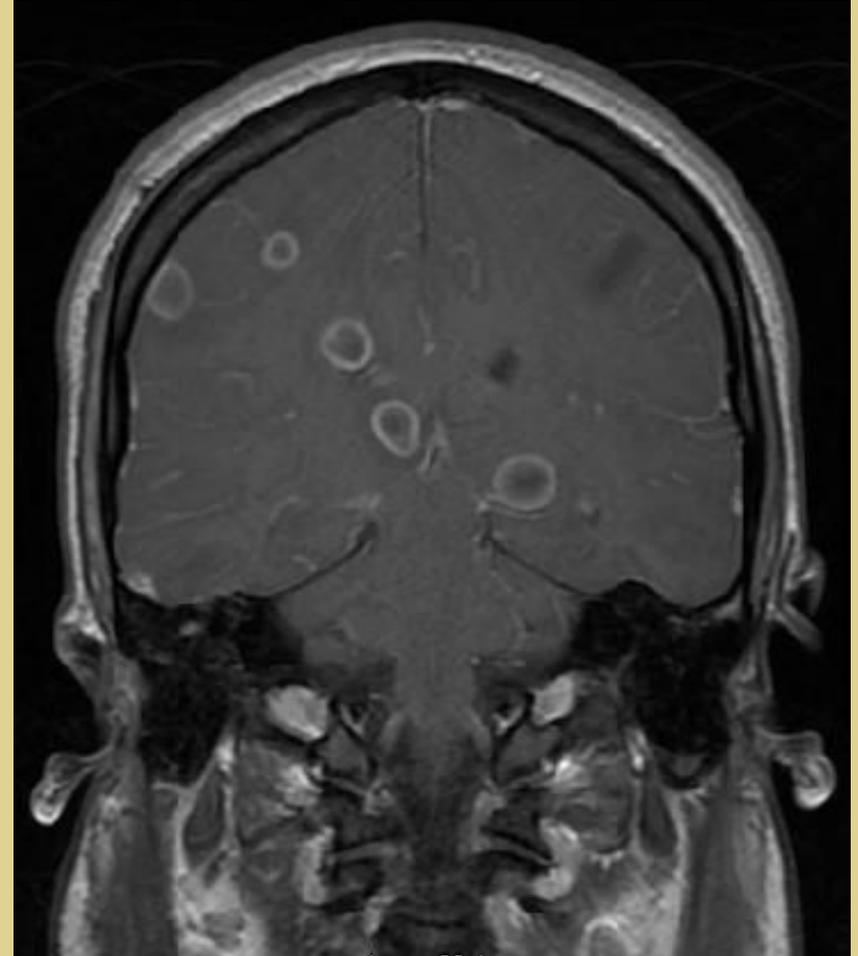
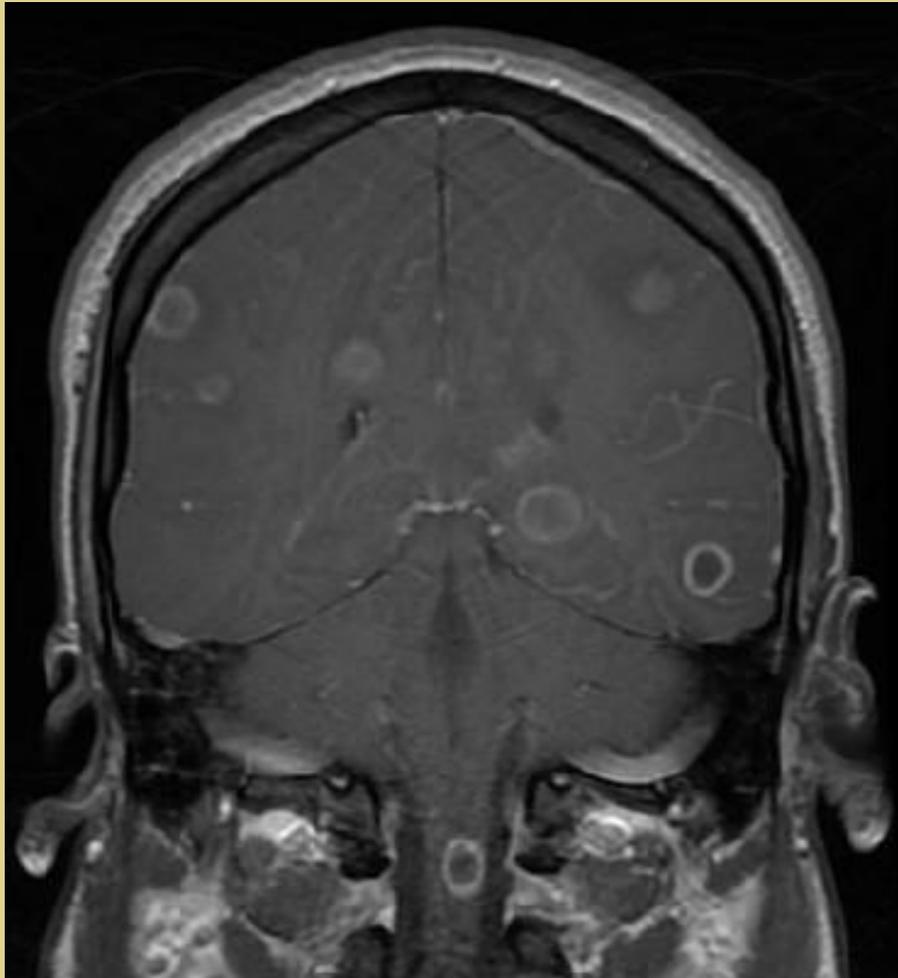
- **\$\$\$\$\$ (450-600K), 4h**
- **Pathogen 99%+ % (depends on genes)**
- **Bug on the plate and in the blood-need DNA**
- **Resistance determinant detected by gene signature; gene diversity --?**
- **New variants +++**
- **Clonal analysis**

Added benefit?? Culture negative

- *Aspergillus* Case report - JCM
- *Streptococcus intermedius* case report –JCM
- 40+ cases of culture negative infection => pathogen detected by PCR/ESI-MS
- 3 cases of recurrent Staph infection confirmed by PCR/ESI-MS
- 6 cases of Strep infection detected by PCR/ESI-MS in patients on antimicrobial treatment

Case 2

- **A previously healthy, HIV negative, immunocompetent 26 year-old male presented with fever, headache, confusion, leukocytosis and bilateral perihilar opacities on CXR.**
 - **CNS imaging was abnormal.**
 - **The CSF WBC count was markedly elevated, but no organisms were detected by gram stain or in culture.**



Case 2

- **PCR/ESI-MS detected *S. intermedius* in both the initial CSF sample obtained by lumbar puncture and a second sample obtained following placement of a ventriculostomy in the right ventricle.**
 - **Following treatment with intravenous ceftriaxone the patient made a complete recovery.**

**No technology will help you
make a clinical (syndrome) diagnosis**

the best tool is between your ears

Thanks!