



Přístupy k detekci antimikrobiální rezistence v praxi

Jak rychle a správně detekovat antibiotickou rezistenci v podmínkách klinické laboratoře

Jaroslav Hrabák

Ústav mikrobiologie a Biomedicínské centrum, Lékařská fakulta v Plzni, Univerzita Karlova

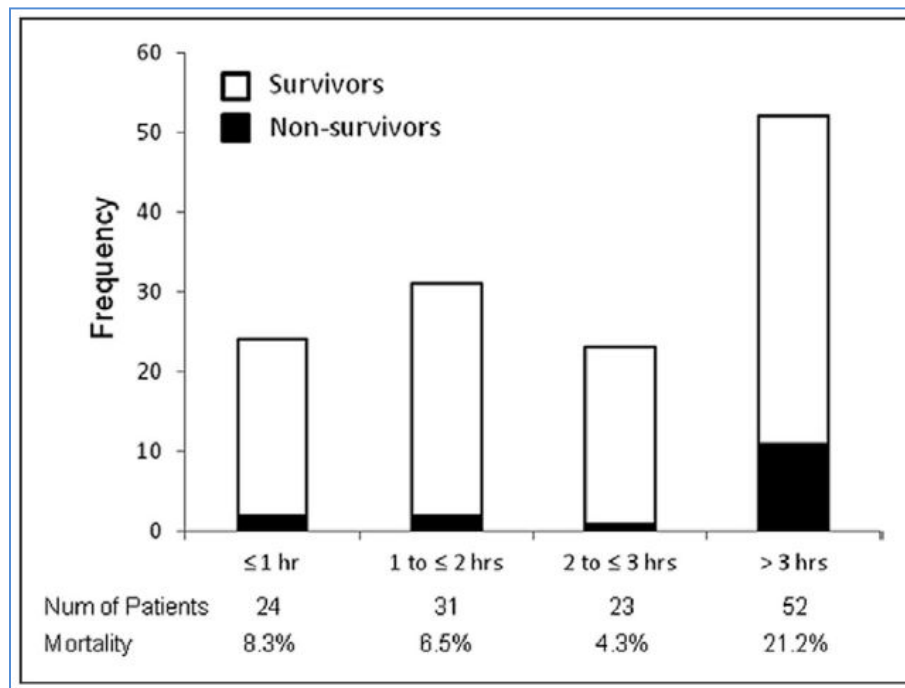
Obsah prezentace

- Jaké jsou přístupy ke klinické rezistenci a jaké jsou definice EUCAST
- Úskalí rychlých metod detekce rezistence
- Příklady rychlých metod v rutinní praxi
- Závěr



Léčba závažných infekčních onemocnění

- Včasné nasazení správné léčby má přímý dopad na zvládnutí infekce



Published in final edited form as:
Crit Care Med. 2014 November ; 42(11): 2409–2417. doi:10.1097/CCM.0000000000000509.

Delayed Antimicrobial Therapy Increases Mortality and Organ Dysfunction Duration in Pediatric Sepsis

Scott L. Weiss, MD¹, Julie C. Fitzgerald, MD, PhD¹, Fran Balamuth, MD, PhD², Elizabeth R. Alpern, MD, MSCE³, Jane Lavelle, MD², Marianne Chilutti, MS⁴, Robert Grundmeier, MD^{4,5}, Vinay M. Nadkarni, MD, MS¹, and Neal J. Thomas, MD, MS⁶



Klinická rezistence a její detekce



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Definice klinické rezistence

- **S - Susceptible, standard dosing regimen:** A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- **I - Susceptible, increased exposure:** A microorganism is categorised as "Susceptible, Increased exposure*" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
- **R - Resistant:** A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.



EUCAST

EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Úskalí definice klinické rezistence



Úskalí definice klinické rezistence

Jak vysoká pravděpodobnost úspěchu / selhání léčby je akceptovatelná?



Rychlá detekce antibiotické rezistence



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Jaká jsou úskalí rychlé detekce rezistence

- Vždy je nutné u rychlých metod zvažovat VME (very major error – chybná interpretace vyšetřovaného izolátu v citlivé kategorii, i když je referenční metodou rezistentní)
- Nezbytné pro detekci rezistence – nasazení vhodné ATB terapie
- Epidemiologické účely
- Deeskalace (???)



Jaká jsou úskalí rychlé detekce rezistence

- Vždy je nutné u rychlých metod zvažovat VME (very major error – chybná interpretace vyšetřovaného izolátu v citlivé kategorii, i když je referenční metodou rezistentní)
- Nezbytné pro detekci rezistence – nasazení vhodné ATB terapie
- Deeskalace (???)
 - Bude ošetřující lékař deeskalovat mimo standardní pracovní dobu?
 - Deeskalace pokud je izolát k antibiotiku volby citlivý (WT)
 - Je etické deeskalovat na antibiotikum, u něhož se projevuje biologická (avšak ne klinická) rezistence (non-wild type) ???



Rychlá detekce antibiotické rezistence

- Kultivační techniky
- Funkční detekce mechanismů rezistence (obvykle enzymatická)
- Detekce buněčných struktur a enzymů zodpovědných za rezistence
- Detekce molekulárních markerů (např. specifické klony, plazmidy)



Kultivační techniky



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Kultivační techniky

- Referenční metoda: **Mikrodiluční bujonová metoda**
(stanovení MIC)
- Všechny kultivační metody musí být validovány oproti standardní metodě (VME, ME)
- Stanovení MIC není modelováním infekce v organismu (!)
- Odpověď bakteriální buňky na antibiotika nemusí být lineární



Kultivační techniky

Stanovení křivek letálního účinku

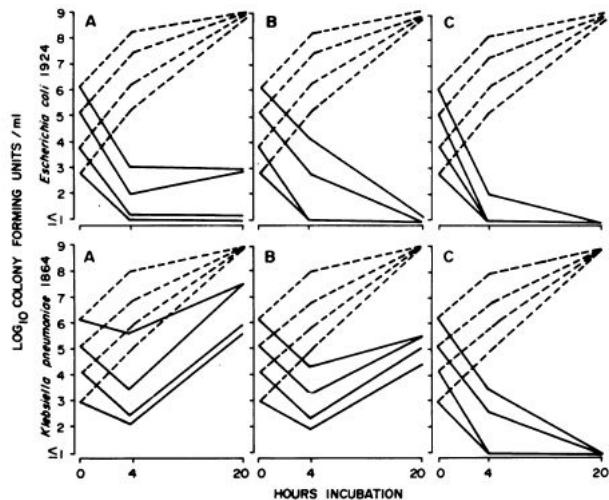


FIG. 1. In vitro killing of *E. coli* 1924 (top) by (A) 16 µg of mecillinam per ml, (B) 16 µg of cefamandole per ml, and (C) both and of *K. pneumoniae* 1864 (bottom) by (A) 16 µg of mecillinam per ml, (B) 16 µg of ceftiofur per ml, and (C) both. Broken line represents growth controls.

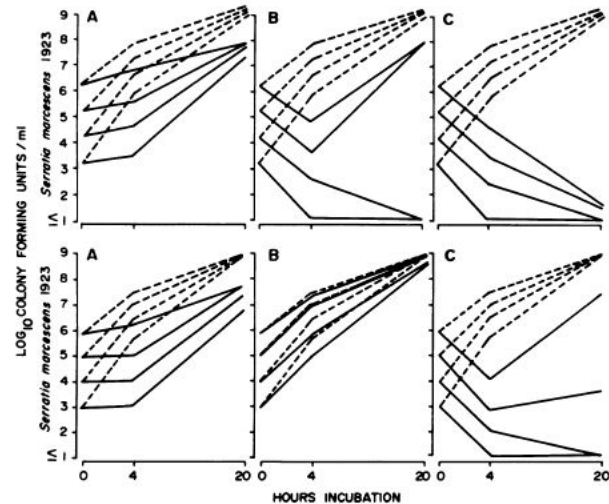


FIG. 2. In vitro killing of *S. marcescens* 1923 (top) by (A) 16 µg of mecillinam per ml, (B) 128 µg of ticarcillin per ml and (C) both and (bottom) by (A) 16 µg of mecillinam per ml, (B) 16 µg of cefamandole per ml, and (C) both. Broken line represents growth controls.

ANTHROPOL AGENTS AND CHEMOTHERAPY, Dec. 1981, p. 905-912
0098-404X/81/12-0905/\$01.00/0

Vol. 18, No. 6

Activity of Mecillinam Alone and in Combination with Other
β-Lactam Antibiotics

ROBERT J. FASS



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Rychlé AST z hemokultur

Journal of
Antimicrobial
Chemotherapy

J Antimicrob Chemother 2020; **75**: 968–978
doi:10.1093/jac/dkz548 Advance Access publication 4 February 2020

The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles

Emma Jonasson^{1*}, Erika Matuschek² and Gunnar Kahlmeter^{1,2}

Table 4. Theoretical and actual numbers of tests, the proportions of tests that could be read and interpreted as S or R after 4, 6 and 8 h and the categorical errors with RAST versus standard DD by EUCAST breakpoint tables version 8.0 at each reading time for all species in NE + SE [RAST breakpoint table version 0 (v. 0) and version 1 (v. 1.0)]

	Incubation time (h)					
	4		6		8	
Breakpoint table	v. 0	v. 1.0	v. 0/v. 1.0	v. 0/v. 1.0	v. 0/v. 1.0	v. 0/v. 1.0
Theoretical number of tests ^a	7024	7361	7361	7361	7361	7361
Number of completed tests ^b	6398	6718	7210	7210	6655	6655
Readable zones ^c (% of completed tests)	5624 (88)	5811 (87)	6921 (96)	6921 (96)	6561 (99)	6561 (99)
Results calculated on readable zones (%)						
breakpoint table	v. 0	v. 1.0	v. 0	v. 1.0	v. 0	v. 1.0
not interpreted as S or R (ATU)	20	16	16	7.5	14	5.7
interpreted as S	71	75	76	84	78	86
interpreted as R	8.8	8.8	7.6	8.5	7.7	8.6
Errors calculated on the total number of zones interpreted as S or R (%)						
breakpoint table	v. 0	v. 1.0	v. 0	v. 1.0	v. 0	v. 1.0
mEs	0.7	0.6	0.5	0.6	0.6	0.8
MEs	2.2	2.1	0.9	1.1	0.7	0.9
VMEs	0.2	0.2	0.4	0.4	0.6	0.5
total errors	3.1	3.0	1.8	2.1	1.8	2.2

Total number of isolates included: $n = 1151$.

Minor error (mE; RAST = S or R and reference method = I); major error (ME; RAST = R and reference method = S); very major error (VME; RAST = S and reference method = R).

^aTotal number of possible isolate/agent combinations. The lower number of tests at 4 h (breakpoint table v. 0) is explained by the absence of norfloxacin breakpoints for *S. aureus*.

^bNumber of completed tests after excluding missing data (e.g. disc dropped).

^cNumber of tests with readable inhibition zones.

Journal of
Antimicrobial
Chemotherapy

J Antimicrob Chemother 2020; **75**: 3230–3238
doi:10.1093/jac/dkaa333 Advance Access publication 13 August 2020

EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories

Anna Åkerlund^{1,2,3*}, Emma Jonasson^{4,5}, Erika Matuschek⁵, Lena Serrander^{2,3}, Martin Sundqvist⁶ and Gunnar Kahlmeter^{4,5} on behalf of the RAST Study Group†



National Institute
of Virology and Bacteriology

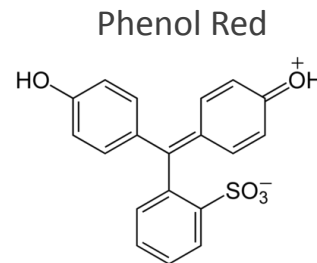
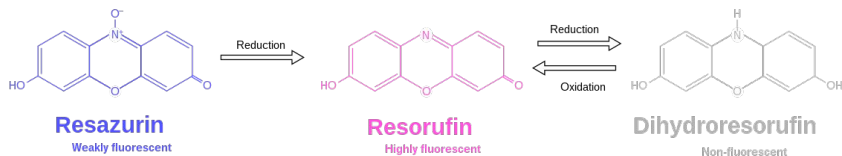


CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Kultivační techniky

Rychlá detekce u specifických antibiotik

- Stanovení optické density během růstu
- Detekce okyselení média (obvyklým substrátem je glukóza)
- Detekce viability (NP testy – resazurin → resorufin)
- Detekce metabolitů



Source: Wikipedia



Kultivační techniky

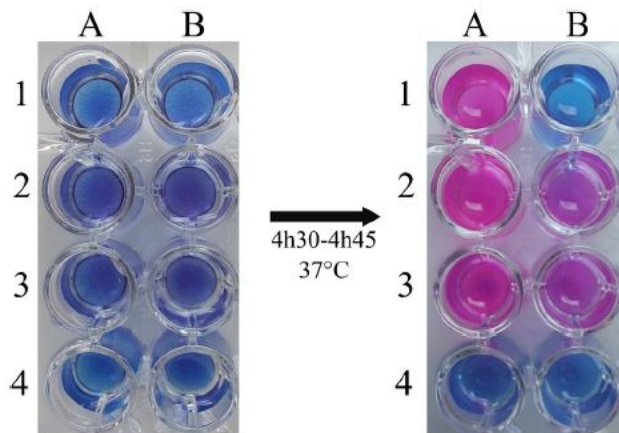
Rychlá detekce u specifických antibiotik

European Journal of Clinical Microbiology & Infectious Diseases (2023) 42:1511–1518
<https://doi.org/10.1007/s10096-023-04691-w>

ORIGINAL ARTICLE

Rapid detection of cefiderocol susceptibility/resistance in *Acinetobacter baumannii*

Otávio Hallal Ferreira Raro¹  · Maxime Bouvier² · Auriane Kerbol² · Jean-Winoc Decousser^{1,4} · Laurent Poirat^{1,2}  · Patrice Nordmann^{1,2,5} 



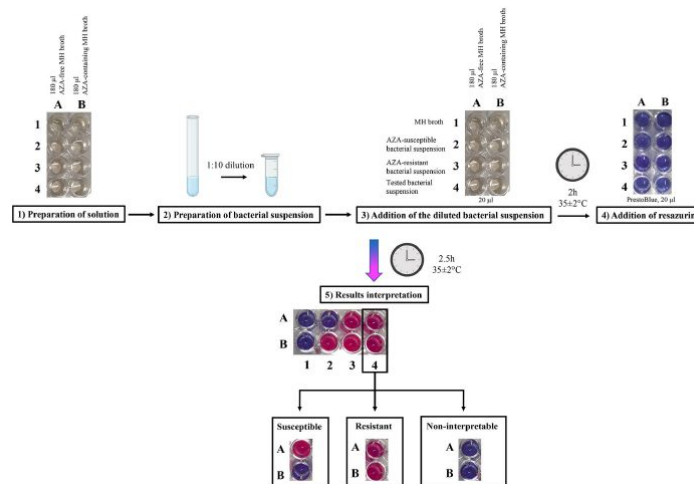
Journal of
Clinical Microbiology



Antimicrobial Chemotherapy | Full-Length Text

Rapid Aztreonam/Avibactam NP test for detection of aztreonam/avibactam susceptibility/resistance in Enterobacterales

Clément Viguer,^{1,2} Maxime Bouvier,^{1,3} Mustafa Sadek,^{1,4} Auriane Kerbol,² Laurent Poirat,^{1,3} Patrice Nordmann^{1,3,4}



National Institute
of Virology and Bacteriology



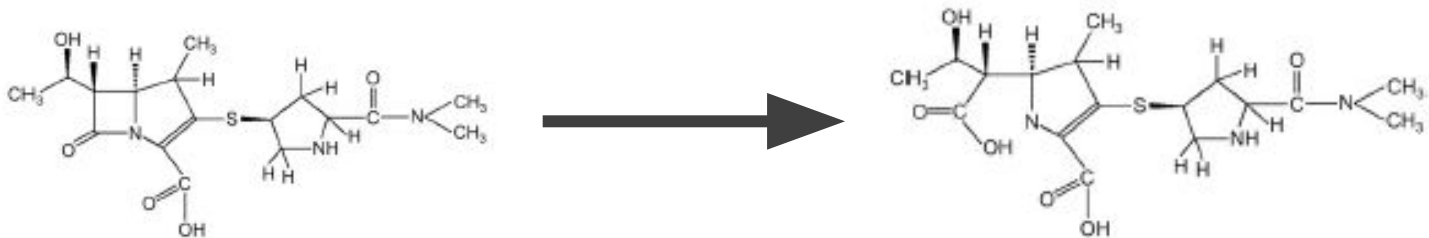
CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Funkční testy mechanismů rezistence



Funkční testy mechanismů rezistence

- Detekce enzymové aktivity
 - Přímá detekce molekuly antibiotika (např. MALDI-TOF MS, spektrofotometrie)
 - Detekce průběhu enzymové reakce (např. změna pH)



Detekce buněčných struktur



National Institute
of Virology and Bacteriology



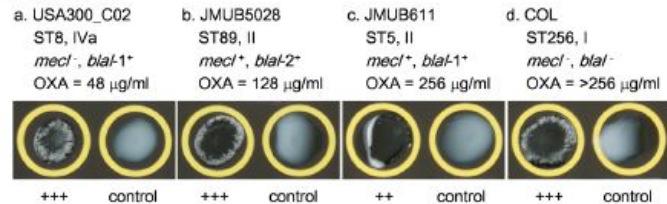
CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Detekce buněčných struktur

- Imunochromatografie
- Detekce MRSA (PBP2a)
- Detekce karbapenemáz

Příklady

1. OR-MRSA



Identification and characterization of mutations responsible for the β -lactam resistance in oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*

Tanit Boonsiri^{1,3}, Shinya Watanabe^{1,3}, Xin-Ee Tan¹, Kanate Thititanapakorn¹, Ryu Narimatsu¹, Kosuke Sasaki², Rami Takenouchi², Yusuke Sato^{1,3}, Yoshifumi Aiba¹, Kotaro Kiga⁴, Teppei Sasahara¹, Yusuke Taki¹, Feng-Yu Li¹, Yuanheng Zhang¹, Aa Haeruman Azam², Tomofumi Kawaguchi² & Longzhu Cui^{1,2}



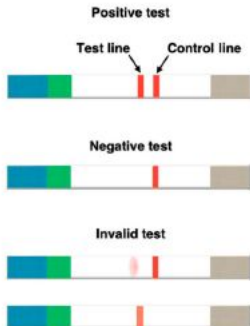
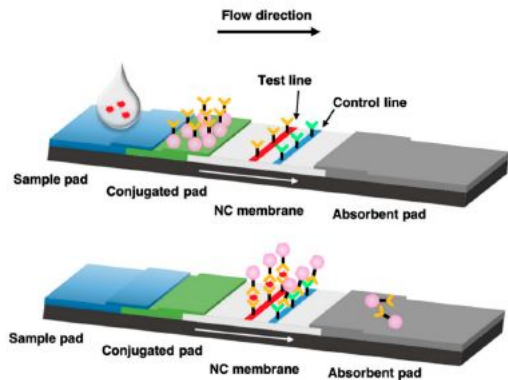
International Journal of
Molecular Sciences



Review

Recent Trends in Lateral Flow Immunoassays with Optical Nanoparticles

Jaehi Kim ¹, Min-Sup Shin ¹, Jonghyun Shin ¹, Hyung-Mo Kim ¹, Xuan-Hung Pham ¹, Seung-min Park ^{2,3}, Dong-Eun Kim ¹, Young Jun Kim ^{1,4} and Bong-Hyun Jun ^{1,*}



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Detekce buněčných struktur

Lekce od ESβLs

- CTX-M-3 vs. CTX-M-15

CTX-M-3: Klasická cefotaximasa – nehydrolyzuje ceftazidim

CTX-M-15: Hydrolysuje cefotaxim i ceftazidim

- Substituce jediné aminokyseliny Asp-240 → Gly**

Journal of Antimicrobial Chemotherapy (2002) 50, 1031–1034
DOI: 10.1093/jac/dkf240

**Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum
β-lactamase CTX-M-15 and of its structurally related β-lactamase
CTX-M-3**

Laurent Poirel¹, Marek Gniadkowski² and Patrice Nordmann^{1*}

JAC

Substrate	CTX-M-15			CTX-M-3		
	k_{cat} (s ⁻¹)	K_m (μM)	k_{cat}/K_m (μM ⁻¹ s ⁻¹)	k_{cat} (s ⁻¹)	K_m (μM)	k_{cat}/K_m (μM ⁻¹ s ⁻¹)
Benzylpenicillin	40	10	4	270	2.5	110
Amoxicillin	20	38	0.5	160	185	1
Ticarcillin	2	5	0.5	40	29	1
Piperacillin	35	13	3	180	66	3
Cefalothin	35	43	0.5	2800	96	30
Cephaloridine	130	83	1.5	130	300	0.5
Cefuroxime	70	13	5	3	49	0.07
Cefoxitin	<0.01	ND	ND	<0.01	ND	ND
Ceftazidime	2	1760	0.001	<0.01	>3000	ND
Ceftriaxone	135	37	3.5	30	58	0.5
Cefotaxime	150	54	3	380	113	3.5
Cefepime	10	1075	0.01	0.2	170	0.001
Cefpirome	120	195	0.6	30	316	0.1
Imipenem	<0.01	ND	ND	<0.01	ND	ND
Aztreonam	1.5	11	0.1	190	188	1



Příklady rychlé detekce klinicky a epidemiologicky závažné rezistence



Detection of Carbapenemases



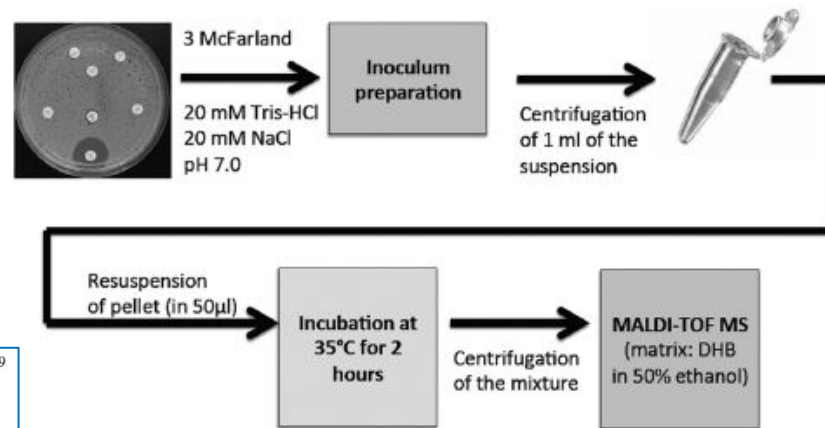
National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

MALDI-TOF MS – detekce β -laktamáz

- Detekce karbapenemáz
- Detekce ES β L a AmpC
- Stanovení typu dle citlivosti k inhibitorům



JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3222–3227
0095-1137/11/\$12.00 doi:10.1128/JCM.00984-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Vol. 49, No. 9

Carbapenemase Activity Detection by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry[▼]

Jaroslav Hrabák,* Radka Walková, Vendula Študentová, Eva Chudáčková, and Tamara Bergerová

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Functional Assay for Rapid Detection of Resistance against β -Lactam Antibiotics

Katrin Sparbier,^a Sören Schubert,^b Ulrich Weller,^c Christiane Boogen,^c and Markus Kostrzewa^a

Bruker Daltonik GmbH, Bremen, Germany^a; Max von Pettenkofer-Institute, Munich, Germany^b; and Praxis für Laboratoriumsmedizin, Ärztliche Gemeinschaft für Diagnostik Köln-Bonn, Cologne, Germany^c

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3321–3324
0095-1137/11/\$12.00 doi:10.1128/JCM.00287-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Vol. 49, No. 9

NOTES

Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry To Detect Carbapenem Resistance within 1 to 2.5 Hours[▼]

Irene Burckhardt* and Stefan Zimmermann

Department for Infectious Diseases, Microbiology and Hygiene, University of Heidelberg,
Im Neuenheimer Feld 324, D-69115 Heidelberg, Germany



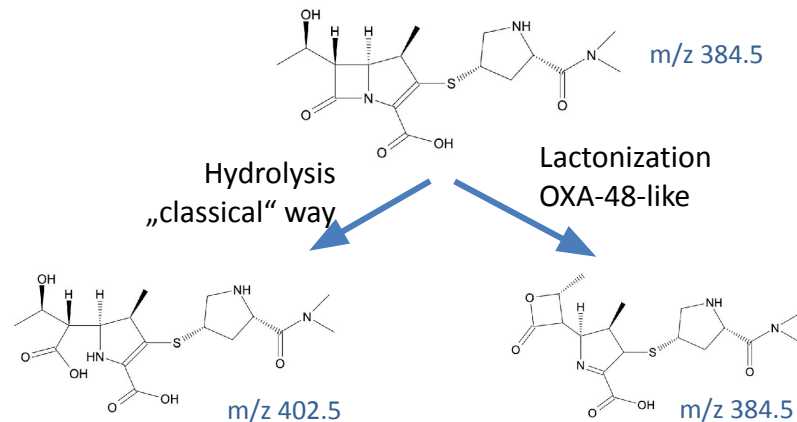
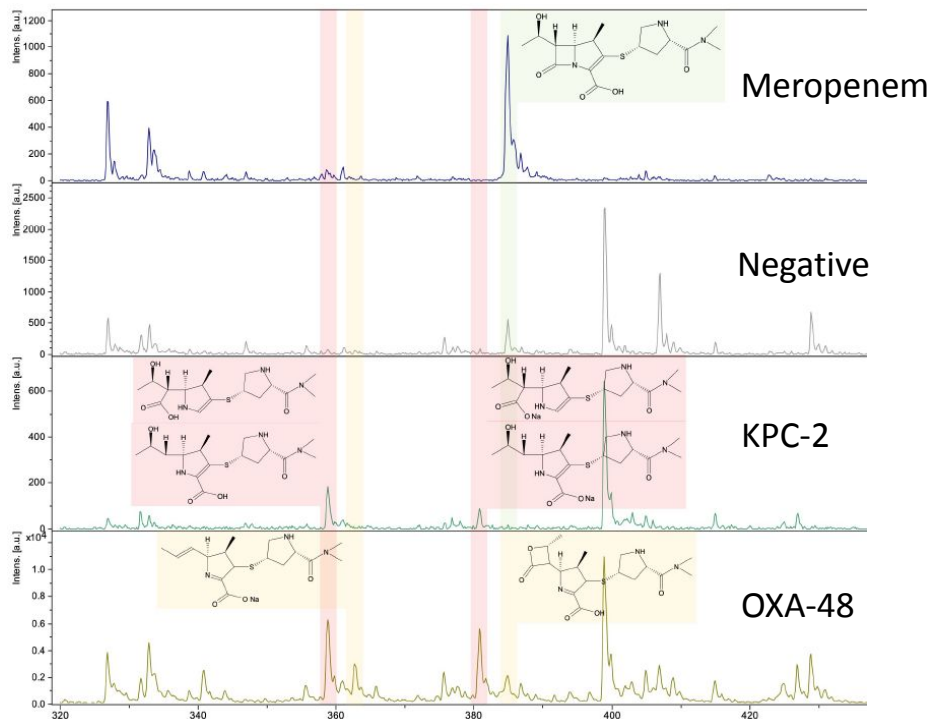
National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

MALDI-TOF MS – detekce β -laktamáz

Specifická detekce enzymů typu OXA-48



Direct identification of OXA-48-type carbapenemases by detection of β -lactone-specific signal using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry



Vendula Studentova, Lucia Dadovska, Jaroslav Hrabak*
Biomedical Centre, Faculty of Medicine in Pilsen, Charles University,
Alej Svobody 76, 323 00 Pilsen, Czech Republic
Department of Microbiology, Faculty of Medicine in Pilsen, Charles
University, Alej Svobody 80, 32300 Pilsen, Czech Republic



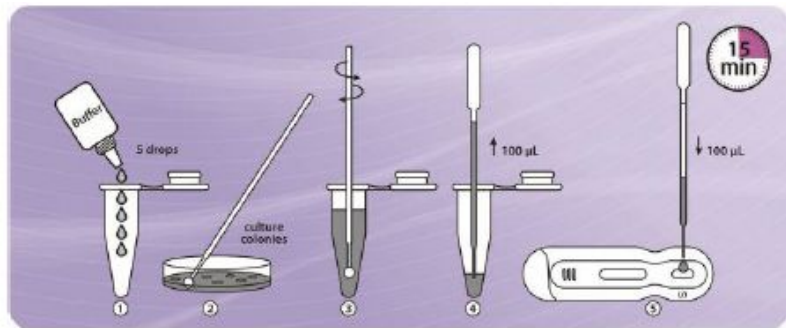
National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Detekce karbapenemáz pomocí imunochromatografie

- Rychlá detekce karbapenemáz 5 nejvýznamnějších skupin (skupiny: KPC, OXA-48-like, NDM, VIM, IMP)
- Negativní výsledek však neznamená, že kmen neprodukuje karbapenemázu! Závisí na lokální epidemiologické situaci.



www.ngbiotech.com

Photo: Kateřina Vlková



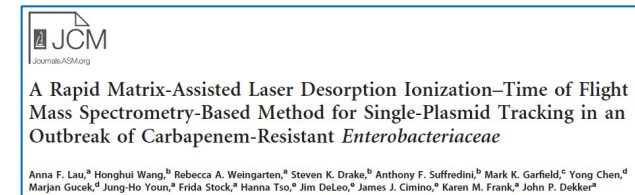
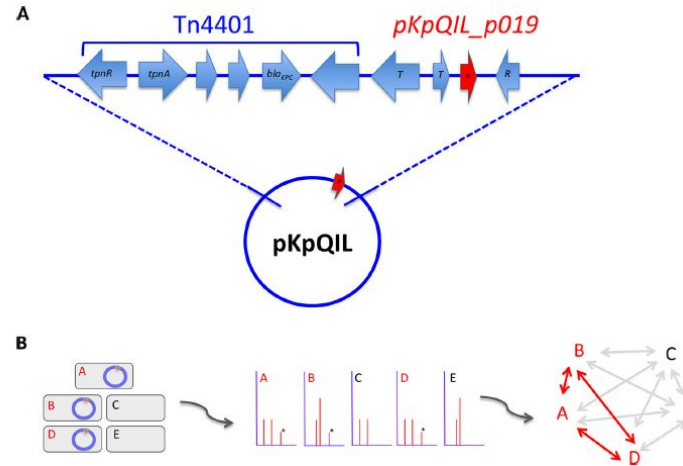
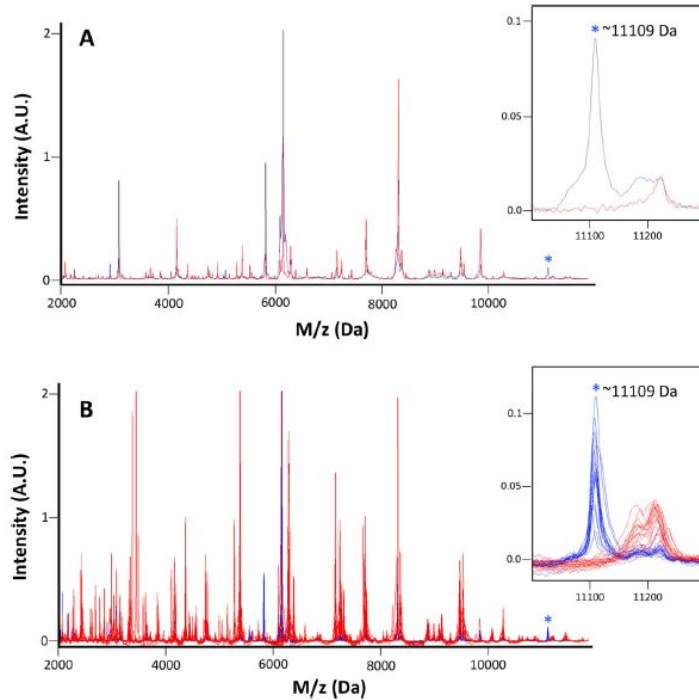
National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

MALDI-TOF MS – Detection of Specific Marker

Detection of putative pKpQIL plasmid

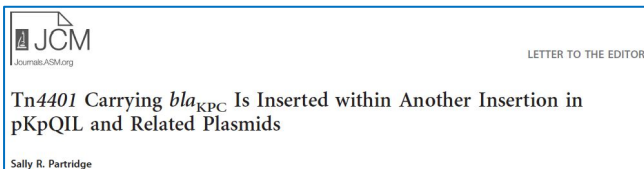


MALDI-TOF MS – Detekce specifických markerů

Detekce pravděpodobného plazmidu pKpQIL

- Nejedná se o detekci karbapenemázy!
- Stejný gen může být nalezen i na jiných plazmidech
- Epidemiologie *bla*_{KPC} je mnohem komplexnější (nejen pKpQIL je zodpovědný za šíření)

producing *Enterobacterales* were recovered from 20 hospitals. Analysis of long-read sequencing data revealed the presence of several types of *bla*_{KPC}-carrying plasmids; 19 out of 25 *bla*_{KPC}-carrying plasmids could be assigned to R (n = 12), N (n = 5), C (n = 1) and P6 (n = 1) incompatibility (Inc) groups. Five of the remaining *bla*_{KPC}-carrying plasmids were multireplicon, while one plasmid couldn't be typed. Additionally, phylogenetic analysis confirmed the spread of *bla*_{KPC}-carrying plasmids among different clones of diverse *Enterobacterales* species. Our findings demonstrated that the increased



Evidence of an epidemic spread of KPC-producing *Enterobacterales* in Czech hospitals

Lucie Kraftová^{1,2}, Marc Finianos^{1,2}, Vendula Studentová^{1,2}, Katerina Chudejová^{1,2}, Vladislav Jakub^{3,4}, Helena Zemlicková^{3,4}, Costas C. Papagiannitsis^{5,6,7,8}, Ibrahim Bitar^{1,2,4,6,7,8} & Jaroslav Hrabak^{1,2}



Nové technologie ve stádiu proof-of-concept



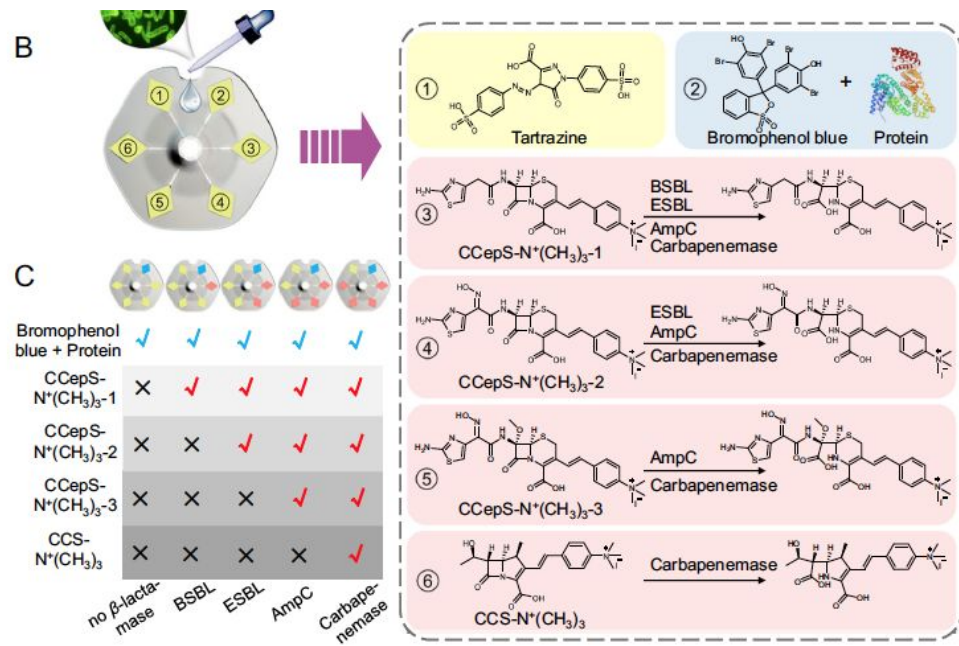
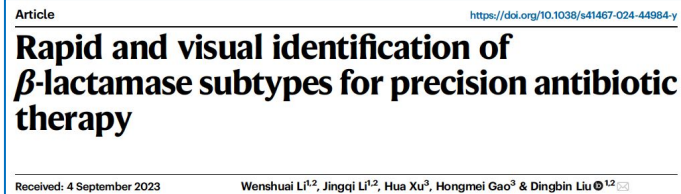
National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Nové techniky - příklady

- V současnosti je vyvíjena řada metod
- Všechny metody však vyžadují precizní validaci



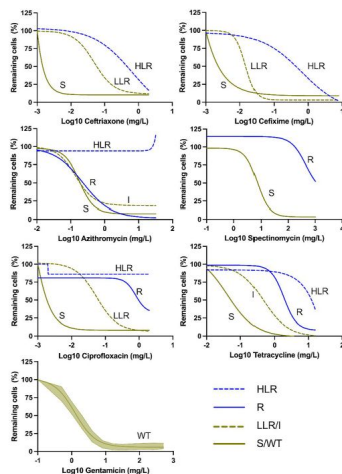
Nové techniky - příklady

J Antimicrob Chemother 2024; 79: 815–819
https://doi.org/10.1093/jac/dkac034 Advance Access publication 9 February 2024

Journal of
Antimicrobial
Chemotherapy

Detection of antimicrobial resistance in <5 h in *Neisseria gonorrhoeae* isolates using flow cytometry—proof of concept for seven clinically relevant antimicrobials

Sofia Somajo¹*, Frida Nilsson², Oskar Ekelund² and Magnus Unemo^{3,4}



nature communications

Article

https://doi.org/10.1038/s41467-024-46273-y

Accurate and rapid antibiotic susceptibility testing using a machine learning-assisted nanomotion technology platform

Received: 1 June 2023

Accepted: 16 February 2024

Published online: 18 March 2024

Check for updates

Alexander Sturm¹, Grzegorz Józwiak¹, Marta Pla Verge², Laura Munch¹, Gino Orlando³, Katja Fromm³, Anthony Vocat³, Amanda Luraschi-Eggemann¹, Clara Orlando³, Katja Fromm³, Eric Delarzo³, Michał Świątkowski¹, Grzegorz Węgliński¹, Roxane M. Tobi³, Maria Garcia-Castillo², Alexandre Delfino³, Florian Taghi³, Sándor Kása^{4,5}, Cornelia Lase-Flohr⁶, Ronald Gietl⁶, Rafael Cantón^{3,7}, Gilbert Greub^{3,8} & Danuta Cichocka^{1,8}

Check for updates

OPEN ACCESS

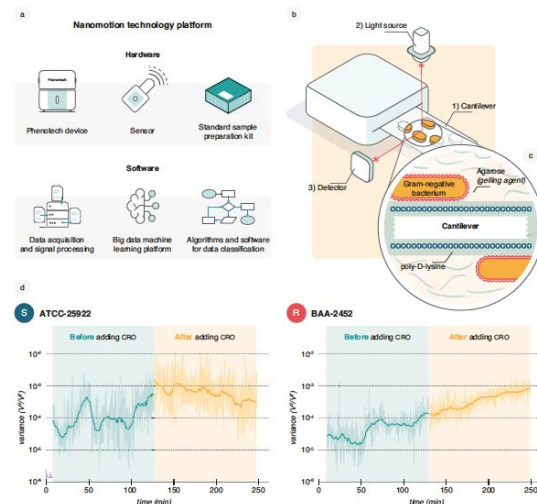
EDITED BY:
Octavio Luiz Franco,
Catholic University of Brasília (UCB), Brazil

REVIEWED BY:
Piyush Baidara,
University of Missouri, United States
Shifu Aggarwal,
Harvard Medical School, United States
ELIDA Nora Ferri,
University of Bologna, Italy

*CORRESPONDENCE
Tanil Kocagoz

A novel rapid bioluminescence-based antimicrobial susceptibility testing method based on adenosine triphosphate consumption

Elif Arık Sever^{1,2}, Esma Aybakan¹, Yeşim Beşli³, Onur Karatuna⁴ and Tanil Kocagoz^{1,5*}



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Závěr



National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

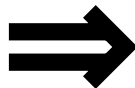
Conclusions

- V současnosti je k dispozici řada metod pro rychlou AST
- Většina metod však není aplikovatelná přímo na klinický vzorek (kromě PCR)
- Všechny diagnostické metody musí být precizně validovány (IVDR legislativa EU)
- Další metody jsou ve stádiu proof-of-concept



Predikce antibiotické rezistence

Detekce mechanismu
rezistence



Predikce klinické
rezistence

Pokud není
mechanismus
rezistence detekován



~~Predikce klinické
citlivosti~~





National Institute
of Virology and Bacteriology



CHARLES UNIVERSITY
Faculty of Medicine in Pilsen