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Identification of CMY-2-Type Cephalosporinases in Clinical Isolates of *Enterobacteriaceae* by MALDI-TOF MS

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This study exploited the possibility to detect *Citrobacter freundii*-derived CMY-2-like cephalosporinases in *Enterobacteriaceae* clinical isolates using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Periplasmic proteins were prepared using a modified sucrose method and analyzed by MALDI-TOF MS. A ca. 39,850-*m/z* peak, confirmed to represent a *C. freundii*-like β-lactamase by in-gel tryptic digestion followed by MALDI-TOF/TOF MS, was observed only in CMY-producing isolates. We have also shown the potential of the assay to detect ACC- and DHA-like AmpC-type β-lactamases.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is increasingly used as a procedure for identification of pathogenic bacteria and fungi due to its time- and cost-effectiveness (1, 2). Recently, further applications of MALDI-TOF MS focusing on antimicrobial resistance mechanisms, including detection of carbapenemase activity in *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp., have been described (3–7).

In 2007, Camara and Hays described for the first time the use of MALDI-TOF MS for differentiating wild-type *Escherichia coli* from ampicillin-resistant (Amp^r) plasmid-transformed *E. coli* strains by the direct visualization of a β-lactamase (8). In a recent MALDI-TOF MS study, Schaumann et al. were not able to distinguish *Enterobacteriaceae* and *P. aeruginosa* isolates producing extended-spectrum β-lactamases (ESBLs) or metallo-β-lactamases (MBLs) from nonproducers (9). Consequently, so far the attempts to visualize native β-lactamases by MALDI-TOF MS in wild-type bacteria have been mostly unsuccessful.

We describe here a new assay for the identification of CMY-2-like β-lactamases in clinical enterobacterial isolates by MALDI-TOF MS. These enzymes are the most prevalent acquired AmpC-type cephalosporinases in *Enterobacteriaceae* (10). The method is based on the extraction of periplasmic proteins and the detection of CMY-2-like β-lactamases by MALDI-TOF MS according to their molecular weight.

Thirty-eight characterized *Enterobacteriaceae* strains from collections of the Faculty of Medicine and University Hospital in Plzen, Czech Republic, the Hellenic Pasteur Institute in Athens, Greece, and the National Medicines Institute in Warsaw, Poland, were used (Table 1) (11, 12). The group included 29 CMY-2-positive clinical isolates, two *E. coli* transconjugants/transformants with CMY-2-like enzymes (*E. coli* A15 or DH5α), and seven non-CMY-producing isolates (13–21). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 were used as negative controls. Purified CMY-2 enzyme (13) was used as a positive control for MALDI-TOF MS measurements.

Isolates were inoculated into 50 ml of Mueller-Hinton broth (Oxoid Ltd.) and incubated at 35°C for 16 h. Cultures were centrifuged at 5,000 × *g* for 20 min, and the cell pellet was used for the extraction of the periplasmic proteins, performed essentially as described by Naglak and Wang (22). Briefly, the pellet was resuspended in 360 μl of 40% sucrose and incubated for 2 h at 4°C. After centrifugation (5,000 × *g*, 5 min), the supernatant was discarded and the cell pellet was resuspended in 360 μl of ice-cold double-distilled water (ddH_2O). After a 30-min incubation at 4°C, 40 μl of 1 M Tris-HCl buffer (pH 7.8) and 12 μl of lysozyme (10 mg/liter) were added to the suspension, which was then incubated for 90 min at 35°C. Spheroplasts were removed by centrifugation (14,000 × *g*, 5 min), leaving the periplasmic fraction in the supernatant.

A 200-μl volume of the periplasmic proteins was added to 1 ml of ice-cold ethanol (95%) supplemented with trifluoroacetic acid (TFA; 0.1%). After 20 min of incubation at −20°C, the solution was centrifuged at 14,000 × *g* for 20 min. The supernatant was removed, and the pellet was allowed to dry. The pellet was resuspended in 50 μl of TFA-acetonitrile-water (0.1:50:49.9 [volume fraction]), using a vortex device for 1 min, and centrifuged (14,000 × *g*, 2 min) to obtain the supernatant extract. Subsequently, 1 μl of each supernatant was applied on a stainless steel MALDI target plate (MSP 96 Target; Bruker Daltonics). After air drying, each sample was overlaid with 1 μl of matrix (sinapinic acid as a saturated solution in 50% ethanol). The matrix/sample spots were allowed to crystallize at room temperature. Each sample was spotted in triplicate. The MALDI-TOF mass spectra were obtained using a Microflex LT mass spectrometer with flexControl 3.3 software (Bruker Daltonics), operating in the positive linear ion mode within the *m/z* range 20,000 to 45,000. The parameters were set up as follows: ion source 1, 20 kV; ion source 2, 16.7 kV; lens, 7 kV; pulsed ion extraction, 170 ns; detection gain, 50×; electronic gain, enhanced (100 mV); sample rate, 2.0 GS/s; mass

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TABLE 1 Summary of the MALDI-TOF MS analysis of the periplasmic extracts[a]

Strain	Country	β-Lactamase(s) produced	Peak at *m/z* 39,850	Reference or source
E. coli pB-cmy2	Greece	Cloned CMY-2	+	13
E. coli S95	Greece	CMY-6	+	14
E. coli S208	Greece	LAT-1, SHV-5, TEM-1	+	14
E. coli T27	Greece	CMY-2, CTX-M-3, TEM-1	−	15
E. coli AK-3281	Greece	CMY-2, TEM-1	+	This study
E. coli AK-5231	Greece	CMY-2, TEM-1	+	This study
E. coli AK-5495	Greece	CMY-2, TEM-1	+	This study
E. coli PL 5143/09	Poland	CMY-2	+	16
E. coli PL 5138/09	Poland	CMY-4	+	16
E. coli PL 6691/10	Poland	CMY-42	+	16
E. coli Cz 9162	Czech Republic	CMY-2, CTX-M-15	+	This study
Trc *E. coli* Cz 9162	Czech Republic	CMY-2	+	This study
E. coli Cz 9178	Czech Republic	CMY-2	+	This study
E. coli Cz 9261	Czech Republic	CTX-M-14	−	This study
E. coli Cz 9309	Czech Republic	CTX-M-27	−	This study
E. coli Cz 9355	Czech Republic	CTX-M-15	−	This study
E. coli A15		NT	−	
E. coli ATCC 25922		NT	−	
E. aerogenes Y15	Greece	CMY-2	+	14
E. aerogenes Y25	Greece	CMY-2, SHV-5	+	14
K. pneumoniae P20	Greece	LAT-1, SHV-5	+	17
K. pneumoniae L67	Greece	CMY-2	−	14
K. pneumoniae N1	Greece	CMY-2, SHV-5, TEM-1	+	14
K. pneumoniae N2	Greece	CMY-2, SHV-5, TEM-1	+	14
K. pneumoniae T80	Greece	CMY-2	+	14
K. pneumoniae HP205	Greece	CMY-36, SHV-5, TEM-1	+	18
K. pneumoniae PL 7246/10	Poland	CMY-2	+	19
K. pneumoniae PL 6185/11	Poland	CMY-4, VIM-19	−	This study
K. pneumoniae Cz 1006	Czech Republic	CMY-2	+	This study
K. pneumoniae Cz 3602	Czech Republic	CMY-2, NDM-1	+	This study
K. pneumoniae Cz 431	Czech Republic	VIM-1, SHV-5	−	This study
K. pneumoniae Cz 597	Czech Republic	KPC-2, OXA-9, SHV-12, TEM-1	−	This study
K. pneumoniae Cz 163243	Czech Republic	SHV-5	−	This study
K. pneumoniae ATCC 13883		NT	−	
P. mirabilis PL 6735/99	Poland	CMY-14, TEM-1	−	20
P. mirabilis PL 27/00	Poland	CMY-12, TEM-2	+	20
P. mirabilis PL 1662/00	Poland	CMY-15, TEM-2	+	20
P. mirabilis PL 864/01	Poland	CMY-4, TEM-1	−	20
P. mirabilis PL 1376/01	Poland	CMY-45, TEM-1	−	20
P. mirabilis PL 1455/04	Poland	CMY-38, TEM-2	+	20
P. mirabilis PM91	Greece	VEB-1, VIM-1	−	21

[a] +, peak at *m/z* 39,850 observed; −, peak at *m/z* 39,850 not observed. Trc, transconjugant; NT, not tested.

range selector, medium range; laser frequency, 30 Hz; digitizer trigger level, 2,500 mV; and laser range, 100%. Spectra were measured manually in at least 10 positions with 500 laser shots. Spectra were analyzed using flexAnalysis 3.0 software (Bruker Daltonics).

The MALDI-TOF MS measurement of the molecular mass of the purified CMY-2 detected one major peak with *m/z* of 39,852 (Fig. 1), slightly differing from the expected value for the mature CMY-2 protein (39,854 [23]). The presence of a peak with a *m/z* of ca. 39,850 was also observed in the mass spectrum of the CMY-2-producing *E. coli* DH5α pB-cmy2 transformant. In the mass spectra of the tested isolates, the ~39,850-*m/z* peak was found in most of the *E. coli* isolates (11/12) and *K. pneumoniae* isolates (8/10) and in all *Enterobacter aerogenes* isolates (2/2) producing CMY-2-like enzymes (Table 1). Of six *Proteus mirabilis* isolates, the peak was identified in three. The latter isolates carried two copies of the bla_{CMY-2}-like gene in their chromosomes, while the false-negative ones carried a single copy of the gene (20). The lack of the ~39,850-*m/z* peak was observed for *E. coli* and *K. pneumoniae* ATCC strains and for all of the non-CMY-producing isolates. Mass spectra of representative isolates are shown in Fig. 1.

The protein content of periplasmic extracts was characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (8). Protein bands of around 40,000 g/mol were detected in extracts of all the CMY-producing strains that were positive in the MALDI-TOF MS assay. These bands comigrated with the purified CMY-2 β-lactamase and were not found in the non-CMY-producing strains and the CMY producers that were nega-

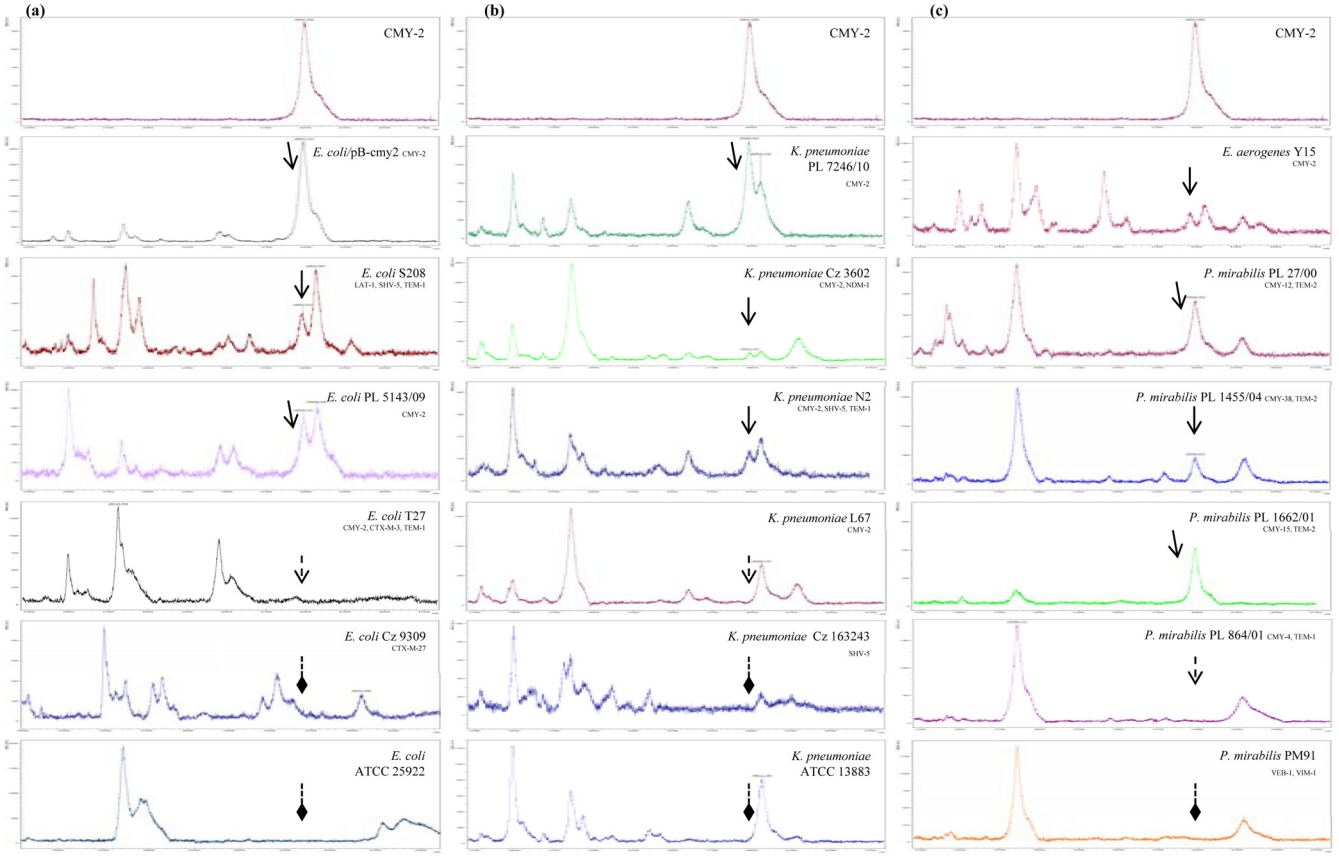

FIG 1 Mass spectra of the purified CMY-2 enzyme and the periplasmic extracts of representative CMY- and non-CMY-producing *E. coli* (a), *K. pneumoniae* (b), and *E. aerogenes* and *P. mirabilis* (c) isolates. Peaks corresponding to CMY β-lactamases are indicated with arrows with solid lines. The absence of the ca. 39,850-*m/z* peaks, representing CMY β-lactamases, is indicated with arrows with dotted lines for CMY producers and diamond-shaped arrows with dotted lines for non-CMY-producing isolates.

tive in the MALDI-TOF MS assay. Identification of proteins observed in SDS-PAGE at approximately 40,000 g/mol was performed by in-gel tryptic digestion followed by MALDI-TOF/TOF MS (24). The identification of the ~40,000-g/mol bands revealed multiple tryptic peptides, being fragments of CMY-2-like polypeptides, for all of the isolates that were positive in the MALDI-TOF MS assay (Table 2). Consistently, such peptides were not detected in extracts from the corresponding gel fragments for all the isolates that were negative by the MALDI-TOF MS assay. The absence of CMY-2-like tryptic peptides in the extracts of CMY producers that were negative in the MALDI-TOF MS assay might be explained by a low concentration of CMY-2-like enzymes in the periplasmic extracts of the respective isolates. However, these results suggested that the presence of the ~39,850-*m/z* peak can be used as an indicator of the presence of the *C. freundii*-derived CMY-2-like group of acquired AmpC β-lactamases (10).

In the preliminary analysis of other AmpC-type β-lactamases, a ca. 39,670-*m/z* peak was observed in the mass spectra of the previously purified ACC-4 enzyme (theoretical relative molecular mass, 39,673 Da) and of the periplasmic extracts of the ACC-4-

TABLE 2 β-Lactamase peptides detected by in-gel tryptic and MALDI-TOF/TOF MS analysis

Observed *m/z* value	Expected *m/z* value	Calculated *m/z* value	ppm	Amino acid sequence[a]	Amino acid position[b]
930.4312	929.4239	929.4032	22.4	K-DYAWGYR-E	217–225
1,285.7499	1,284.7426	1,284.7150	21.5	K-TLQQGIALAQSR-Y	266–279
1,544.8279	1,543.8206	1,543.7895	20.1	K-SYPNPVRVEAAWR-I	362–376
1,556.8695	1,555.8622	1,555.8318	19.6	-AAKTEQQIADIVNR-T	21–35
1,658.8035	1,657.7962	1,657.7518	26.8	R-WVQANMDASHVQEK-T	252–267
1,664.9054	1,663.8981	1,663.8682	18.0	K-LAHTWITVPQNEQK-D	203–218
1,827.1019	1,826.0946	1,826.0665	15.4	K-VALAALPAVEVNPPAPAVK-A	310–330
2,081.1052	2,080.0979	2,080.0589	18.8	R-EGKPVHVSPGQLDAEAYGVK-S	224–245

[a] Hyphens indicate tryptic restriction sites. Small capital letters correspond to amino acids found outside the restriction sites. Underlined residues were modified by oxidation.
[b] CMY-2 peptide from *K. pneumoniae* HEL-1 (GenBank accession no. CAA62957) was used as a reference for the alignment of β-lactamase tryptic peptides.

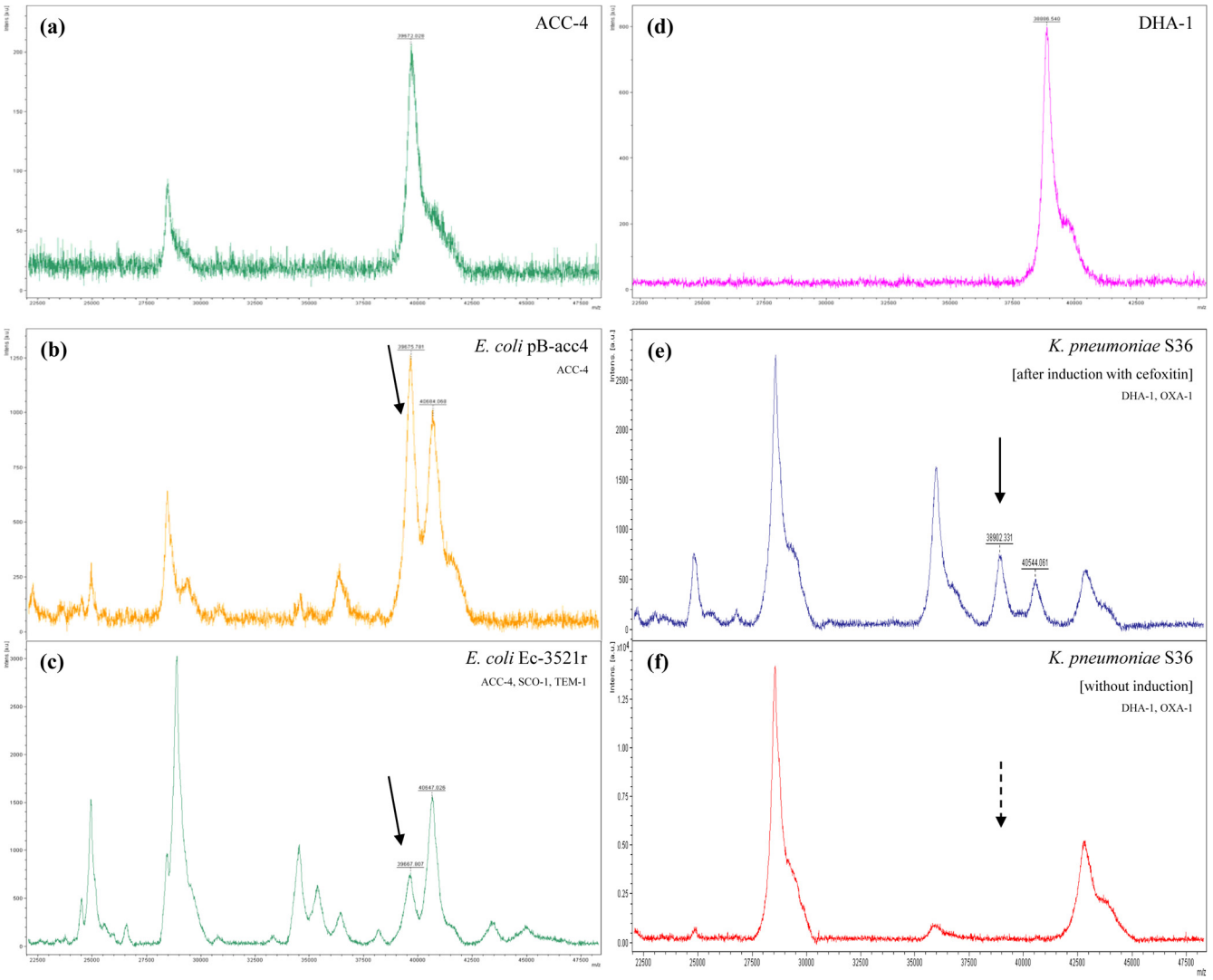

FIG 2 (a to c) Mass spectra of the purified ACC-4 enzyme (a) and the periplasmic extracts of ACC-producing *E. coli* pB-acc4 (b) and EC-3521r (c). (d to f) Mass spectra of the purified DHA-1 enzyme (d) and the periplasmic extracts of DHA-producing *K. pneumoniae* S36 after induction with cefoxitin (e) and without induction (f). Peaks corresponding to β-lactamases are indicated with arrows with solid lines. In the mass spectra of *K. pneumoniae* S36, the dotted arrow indicates the absence of the ca. 38,900-*m*/*z* peak, representing DHA-1 β-lactamase, in periplasmic extract prepared without adding cefoxitin in broth culture.

producing *E. coli* EC-3521r and pB-acc4/DH5α strains (Fig. 2) (25). The MALDI-TOF MS measurement of the molecular mass of the purified DHA-1 β-lactamase detected a 38,887-*m*/*z* peak (theoretical relative molecular mass, 38,881 Da). In the DHA-1-producing isolate *K. pneumoniae* S36 strain (26) with the functional *ampC-ampR* system (10), a corresponding peak of ca. 38,900-*m*/*z* was observed but only when the AmpC production was induced by adding cefoxitin at 50 μg/ml in broth cultures 3 h before harvesting the cells (Fig. 2). These data suggested that the assay can be used for the detection of other AmpC-type β-lactamases. Additionally, the observation of the 39,850-*m*/*z*, ~39,670-*m*/*z*, and ~38,900-*m*/*z* peaks for CMY-2-like, ACC-4, and DHA-1 enzymes, respectively, indicated that MALDI-TOF MS may discriminate the diverse groups of acquired AmpC-type cephalosporinases.

In this study, we showed for the first time that MALDI-TOF MS has the potential to detect the most clinically important acquired AmpC β-lactamases, such as the CMY-2-like, ACC, and DHA types, in clinical isolates of *Enterobacteriaceae*. The described MALDI-TOF MS assay worked well with most of the CMY-producing isolates. However, the method performed poorly for *P. mirabilis*. It might be hypothesized that, in that case, increased production of a CMY-2-like enzyme upon gene duplication is important for the visualization of the ~39,850-*m*/*z* peak.

In agreement with previous studies illustrating that MALDI-TOF MS applications are quick and cheap procedures (3), the described protocol exhibits an 22-h turnaround time, which is comparable to that of molecular techniques only if considering PCR plus sequencing of the amplicon in order to identify the specific allelic variant of the β-lactamase gene. The use of classic PCR and real-time PCR (RT-PCR) assays in clinical settings is more expensive than the use of the described MALDI-TOF (not

considering the initial cost of investment for the equipment) but is less labor intensive and with a shorter turnaround time. Detection of β-lactamases by MALDI-TOF MS is a proteomic approach allowing the study of the behavior of the tested strains and should complement techniques already used for characterization of β-lactamases such as PCR and isoelectric focusing (IEF). The fact that MALDI-TOF MS can directly detect class A (9) and class C β-lactamases, as well as other mechanisms such as methylation of rRNA and cell wall components (3, 27), indicates the feasibility of establishing a MALDI-TOF supplementary database of resistance mechanisms that would promote research in this field. Notwithstanding the aforementioned problems, we strongly believe that proper modifications and validation of the described MALDI-TOF assay will easily lead to acceptance of its future application in diagnostic laboratories and reference centers.

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A patent application corresponding to this test has been sent on behalf of Charles University.

REFERENCES

1. **Seng P, Rolain JM, Fournier PE, La Scola B, Drancourt M, Raoult D.** 2010. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol. **5:**1733–1754. http://dx.doi.org/10.2217/fmb.10.127.
2. **Wieser A, Schneider L, Jung J, Schubert S.** 2012. MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl. Microbiol. Biotechnol. **93:**965–974. http://dx.doi.org/10.1007/s00253-011-3783-4.
3. **Hrabák J, Chudácková E, Walková R.** 2013. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. Clin. Microbiol. Rev. **26:**103–114. http://dx.doi.org/10.1128/CMR.00058-12.
4. **Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T.** 2011. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **49:**3222–3227. http://dx.doi.org/10.1128/JCM.00984-11.
5. **Burckhardt I, Zimmermann S.** 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J. Clin. Microbiol. **49:**3321–3324. http://dx.doi.org/10.1128/JCM.00287-11.
6. **Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunnel JM, Drissi M, Msli E, Touati A, Rolain JM.** 2012. Rapid detection of carbapenem resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One **7:**e31676. http://dx.doi.org/10.1371/journal.pone.0031676.
7. **Hrabák J1, Studentová V, Walková R, Zemlicková H, Jakubu V, Chudácková E, Gniadkowski M, Pfeifer Y, Perry JD, Wilkinson K, Bergerová T.** 2012. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapnemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **50:**2441–2443. http://dx.doi.org/10.1128/JCM.01002-12.
8. **Camara JE, Hays FA.** 2007. Discrimination between wild-type and ampicillin-resistant *Escherichia coli* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Bioanal. Chem. **389:**1633–1638. http://dx.doi.org/10.1007/s00216-007-1558-7.
9. **Schaumann R, Knoop N, Gnzel GH, Losensky K, Rosenkranz C, Stîngu CS, Schellenberger W, Rodloff AC, Eschrich K.** 2012. A step towards the discrimination of beta-lactamase-producing clinical isolates of Enterobacteriaceae and Pseudomonas aeruginosa by MALDI-TOF mass spectrometry. Med. Sci. Monit. **18:**MT1–MT77. http://dx.doi.org/10.12659/MSM.883339.
10. **Jacoby GA.** 2009. AmpC beta-lactamases. Clin. Microbiol. Rev. **22:**161–182. http://dx.doi.org/10.1128/CMR.00036-08.
11. **Empel J, Baraniak A, Literacka E, Mrówka A, Fiett J, Sadowy E, Hryniewicz W, Gniadkowski M; Beta-PL Study Group.** 2008. Molecular survey of beta-lactamases conferring resistance to newer beta-lactams in *Enterobacteriaceae* isolates from Polish hospitals. Antimicrob. Agents Chemother. **52:**2449–2454. http://dx.doi.org/10.1128/AAC.00043-08.
12. **Pérez-Pérez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40:**2153–2162. http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002.
13. **Kotsakis SD, Papagiannitsis CC, Tzelepi EE, Tzouvelekis LS, Miriagou V.** 2009. Extended-spectrum properties of CMY-30, a Val211Gly mutant of CMY-2 cephalosporinase. Antimicrob. Agents Chemother. **53:**3520–3523. http://dx.doi.org/10.1128/AAC.00219-09.
14. **Gazouli M, Tzouvelekis LS, Prinarakis E, Miriagou V, Tzelepi E.** 1996. Transferable cefoxitin resistance in enterobacteria from Greek hospitals and characterization of a plasmid-mediated group 1 beta-lactamase (LAT-2). Antimicrob. Agents Chemother. **40:**1736–1740.
15. **Mavroidi A, Tzelepi E, Miriagou V, Gianneli D, Legakis NJ, Tzouvelekis LS.** 2002. CTX-M-3 beta-lactamase-producing *Escherichia coli* from Greece. Microb. Drug Resist. **8:**35–37. http://dx.doi.org/10.1089/10766290252913737.
16. **Izdebksi R, Baraniak A, Fiett J, Adler A, Kazma M, Salomon J, Lawrence C, Rossini A, Salvia A, Hryniewicz W, Brun-Buisson C, Carmeli Y, Gniadkowski M; MOSAR WP2 and WP5 Study Groups.** 2013. Clonal structure, extended-spectrum β-lactamases, and acquired AmpC-type cephalosporinase of *Escherichia coli* populations colonizing patients in rehabilitation centers in four countries. Antimicrob. Agents Chemother. **57:**309–316. http://dx.doi.org/10.1128/AAC.01656-12.
17. **Tzouvelekis LS, Tzelepi E, Mentis AF.** 1994. Nucleotide sequence of a plasmid-mediated cephalosporinase gen (bla_{LAT-1}) found in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **38:**2207–2209. http://dx.doi.org/10.1128/AAC.38.9.2207.
18. **Zioga A, Whichard JM, Kotsakis SD, Tzouvelekis Tzelepi LS E, Miriagou V.** 2009. CMY-31 and CMY-36 cephalosporinases encoded by ColE1-like plasmids. Antimicrob. Agents Chemother. **53:**1256–1259. http://dx.doi.org/10.1128/AAC.01284-08.
19. **Baraniak A, Izdebski R, Fiett J, Sadowy E, Adler A, Kazma M, Salomon J, Lawrence C, Rossini A, Salvia A, Vidal Samso J, Fierro J, Paul M, Lerman Y, Malhotra-Kumar S, Lammens C, Goossens H, Hryniewicz W, Brun-Buisson C, Carmeli Y, Gniadkowski M; MOSAR WP2 and WP5 Study Groups.** 2013. Comparative population analysis of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases colonizing patients in rehabilitation centers in four countries. Antimicrob. Agents Chemother. **57:**1992–1997. http://dx.doi.org/10.1128/AAC.02571-12.
20. **D'Andrea MM, Leteracka E, Zioga A, Giani T, Baraniak A, Fiett Sadowy JE, Tassios PT, Rossolini GM, Gniadkowski M, Miriagou V.** 2011. Evolution and spread of a multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type cephalosporinase in Europe. Antimicrob. Agents Chemother. **55:**2735–2742. http://dx.doi.org/10.1128/AAC.01736-10.
21. **Papagiannitsis CC, Miriagou V, Kotsakis SD, Tzelepi E, Vatopoulos AC, Petinaki E, Tzouvelekis LS.** 2012. Characterization of a transmissible plasmid encoding VEB-1 and VIM-1 in *Proteus mirabilis*. Antimicrob. Agents Chemother. **56:**4024–4025. http://dx.doi.org/10.1128/AAC.00470-12.
22. **Naglak TJ, Wang HY.** 1990. Recovery of a foreign protein from the periplasm of *Escerichia coli* by chemical permeabilization. Enzyme Microb. Technol. **12:**603–611. http://dx.doi.org/10.1016/0141-0229(90)90134-C.
23. **Kotsakis SD, Caselli E, Tzouvelekis LS, Petinaki E, Prati F, Miriagou V.** 2013. Interactions of oximino-substituted boronic acids and β-lactams with the CMY-2-derived extended-spectrum cephalosporinases CMY-30 and CMY-42. Antimicrob. Agents Chemother. **57:**968–976. http://dx.doi.org/10.1128/AAC.01620-12.
24. **Mares J, Richtrova P, Hricinova A, Tuma Z, Moravec J, Jysak D, Matejovic M.** 2010. Proteomic profiling of blood-dialyzer interactome reveals involvement of lectin complement pathway in hemodialysis-

induced inflammatory response. Proteomics Clin. Appl. **4:**829–838. http://dx.doi.org/10.1002/prca.201000031.

25. **Papagiannitsis CC, Tzouvelekis LS, Tzelepi E, Miriagou V.** 2007. Plasmid encoded ACC-4, an extended-spectrum cephalosporinase variant from *Escherichia coli*. Antimicrob. Agents Chemother. **51:**3763–3767. http://dx.doi.org/10.1128/AAC.00389-07.
26. **Empel J, Hrabak J, Kozinska A, Bergerova T, Ubraskova P, Kern-Zdanowicz I, Gniadkowski M.** 2010. DHA-1-producing *Klebsiella pneumoniae* in a teaching hospital in the Czech Republic. Microb. Drug Resist. **16:**291–295. http://dx.doi.org/10.1089/mdr.2010.0030.
27. **Cai JC, Hu YY, Zhang R, Zhou HW, Chen GX.** 2012. Detection of OMPK36 proin loss in *Klebsiella* spp. by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **50:**2179–2182. http://dx.doi.org/10.1128/JCM.00503-12.