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Fast and easy detection of CMY-2 in *Escherichia coli* by direct MALDI-TOF Mass Spectrometry

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Running title:Discrimination of CMY-2-producing *E. coli* by MALDI-TOF

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Abstract

Fast typing methods for third generation cephalosporin resistance mechanisms are needed to guide appropriate treatment and prevent potential dissemination events. In this study we used a novel short and fast methodology for the identification of CMY-2 in 50 well characterized clinical isolates of *E. coli* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry- MALDI-TOF MS. Samples were prepared using the double layer sinapinic acid technique for detection of intact proteins Comparison among mass spectral profile of different strains between m/z 35,000 – 45,000 Da showed that two groups of isolates could be differentiated after peak analysis. A single distinctive peak with different intensities, at approximately m/z 39,800 Da was found in all CMY-2 producing strains (transconjugant, transformant and wild type) and consistently absent in the control groups (ESBL producers and susceptible strains). Statistical results showed 100 % values for sensitivity and specificity, indicating a perfect test and a high discriminative power. In this study, we demonstrated that MALDI-TOF MS has the potential to detect directly the most clinically relevant acquired AmpC β-lactamase, the CMY-2-enzyme, in *E. coli* with a less time-consuming process as compared to conventional methods. Our results may constitute the basis for further research to detect other β-lactamases, or even other resistance markers.

Keywords: CMY-2; *Escherichia coli*; MALDI-TOF; β-lactamase detection

1 Introduction

Resistance to third generation cephalosporins (TGC) is one of the most relevant problems in current Gram–negative antimicrobial therapy, leading to overuse of carbapenems and emergence of different carbapenemases. Outstanding from others, different plasmid borne extended spectrum β-lactamases (ESBL) families and plasmid-determined AmpC-type β-lactamases are the most relevant resistance mechanisms. Among them, CTX-M-type and CMY-2 β-lactamases are the most frequent resistance markers worldwide, respectively (Gutkind et al., 2013).

Phenotypic methods, as double disk synergy tests using β-lactam and β-lactamase-inhibitor disks, and even the original double disk approximation tests remain convenient, easy to perform, but not fast methods for detecting these β-lactamase in Gram-negative bacilli.

Clinical microbiology laboratories are expected nowadays to provide a proper characterization of the involved mechanisms for an effective antimicrobial therapy (Morency-potvin et al., 2017). Fast typing methods for third generation cephalosporin (TGC) resistance mechanisms are necessary to guide an appropriate antimicrobial treatment scheme and prevent potential dissemination events (Rhodes et al., 2017). This is also the case for any resistance mechanism, and different genotypic systems have been developed for achieving this goal.

Incorporation of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been one of the most significant breakthrough technologies that re defined modern clinical microbiology, and is today routinely used in high complexity laboratories for rapid species identification (Sandalakis et al., 2017). Recent studies demonstrated the possibility of using MALDI-TOF MS approach to

predict β-lactamase production based on the detection of hydrolyzed β-lactam substrates. This hydrolysis is evaluated after at least one hour of incubation in the presence of the bacteria (Hooff et al., 2012; Oviaño et al., 2016; Sparbier et al., 2012). Following this methodology, enzyme detection is feasible but only reflects the presence of at least one enzyme with hydrolytic activity on the provided substrate.

Direct detection of a specific enzyme by analysis of the MALDI-TOF MS generated protein profile has been the subject of research in different studies. Among them, Camaro and Hays were the first to detect a peak corresponding to the β-lactamase TEM-1, analyzing the spectrum of proteins in bacterial lysates of *E. coli* susceptible and resistant to ampicillin (Camara and Hays, 2007). More recently, Papagiannitsis *et al* detected CMY-2 enzyme in different enterobacteria after extraction of periplasmic proteins using a multiple steps procedure (Hart et al., 2015; Papagiannitsis et al., 2014).

Antibiotic resistance has been predicted by MALDI-TOF in the identification mass range by detection of biomarkers associated with a specific resistance determinant. In this respect, the hypothetical protein named p019 (11.109 Da) linked to the transposon Tn*4401*a was used to predict KPC carbapenemase producers (Gaibani et al., 2016; Youn et al., 2016). In *Staphylococcus aureus* the peptide PSM-mec (2.413 Da) was associated with the methicillin resistance (Rhoads et al., 2016). Detection of these markers is relevant if only very conserved platforms are associated to the resitance marker. On the other hand, direct intact β-lactamase detection can be directly associated to protein expression, inferring the resistance patterns of bacteria.

In the present study, we describe an easy and quick methodology for the identification of mature CMY-2 β-lactamase by MALDI-TOF in clinical isolates of *Enterobacterales* lacking inducible chromosomal *ampC* gene, mainly *E. coli*. The

method is based on total protein extraction analysis using an organic solvent, and further detection of the mature protein given its theoretical molecular weight (This material was partially presented at ASM Microbe 2017, New Orleans).

2 Materials and Methods

2.1 Bacterial strains and β-lactamases characterization

Sixty three previously characterized *Enterobacterales* clinical strains (50 *E. coli, 4 Klebsiella pneumoniae*, 3 *Proteus mirabilis* and 6 *Salmonella* spp.), deposited at our laboratory collection in Buenos Aires, Argentina, were included (Table 1). Even if the initial attempts were done on *bona fide* transconjugant, transformant and recipient strains, finally 29 unrelated CMY-2 producing clinical isolates of *E. coli* and 21different $bla_{\text{CMY-2}}$ negative *E. coli* isolates were incorporated. There was no epidemiological link among these isolates. Other species of CMY-2-producing *Enterobacterales* were also tested (Table 1). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were also included as negative controls. All isolates were previously characterized both phenotypically (by disk diffusion method and synergy test) and genotypically (by PCR and sequencing) aimed at different β-lactamase gene families (CMY-, TEM-, CTX-M-). Assays were performed as already described (Cejas et al., 2012; Rincón Cruz et al., 2013; Saba Villarroel et al., 2017). As test material, cultures were obtained on Mueller Hilton (MH) (Britania, Argentina) agar plates without antibiotics (for all susceptible and resistant isolates) or in MH agar plates supplemented with ampicillin (Sigma-Aldrich, USA) 100 mg/L (for all resistant isolates), incubated overnight at 37°C.

2.2 Sample preparation for MALDI-TOF MS

The organic solvent extraction method was fine-turned in this work in order to achieve at total protein analysis. This procedure was adapted from those proposed by

Matsuda *et al* (Matsuda et al., 2012). Briefly, a loopful of bacterial colonies (4-5 colonies) was suspended in 300 µl distilled water and mixed by vortexing for 30 s. After that 900 µl of room temperature absolute ethanol (Sigma-Aldrich, USA) were added. The suspension was vortexed vigorously for 30 s and centrifuged at 17,000 x *g* for 2 min at room temperature. After discarding the supernatant, the pellet was re-suspended in 100 µl of extraction solvent (formic acid - isopropyl alcohol - water, 17:33:50 ratio by volume) (Sigma-Aldrich, USA), vortexed for 30 s, and then centrifuged at 17,000 x *g* for 2 min at room temperature to obtain the clean supernatant extract.

2.3 Target spot loading

The double layer sinapinic acid (SA) (Bruker Daltonics, Germany) technique was used for intact protein detection. To form the first layer, 0.7 µl of a saturated solution of SA in absolute ethanol was loaded onto a MALDI target spot and dried at room temperature. As a second layer, the protein extracts were mixed 1:1 with SA 10 g/L solution (30:70 [v/v] acetonitrile: 0.1% trifluoroacetic acid in water) (Sigma-Aldrich, USA). One µL of the sample/matrix mixture was then deposited onto the first layer. Samples were dried at room temperature before being placed in the mass spectrometer (Keller and Li, 2006). Each sample was analyzed by triplicate, loaded three times on different spots of the stainless steel MALDI target plate.

2.4 Spectra acquisition and statistical analysis

The mass spectra were obtained using a Microflex LT mass spectrometer by flexControl3.4 software (Bruker Daltonics, Germany). The parameters were set up as follows: linear positive ion mode within the mass range of 17,000 Da to 50,000 Da. Spectrometer ion source 1, 19.98 kV; ion source 2, 17.93 kV; lens 5.50 kV; pulsed ion extraction 260 ns; detection gain, 2877 V; sample rate and electronic setting 0.50 GS/s; High mass range. Laser frequency 60 Hz and laser range between 80-100%.

Spectra of each spot were captured twice in automatic mode. Each spectrum was obtained after 800 shots (16×50 laser shots for random walk in partial sample) per spot. Data were automatically acquired using AutoXecute acquisition control software (Bruker Daltonics, Germany). Spectra were analyzed using flexAnalysis 3.4 software (Bruker Daltonics). Before each run, Microflex LT mass spectrometer was externally calibrated using the Standard II calibration mixture (Bruker Daltonics).

All spectra were assessed with the software ClinProTools 3.0 (Bruker Daltonics, Germany) (Ketterlinus et al., 2005).

2.5 Mass spectrometry analysis

In order to confirm the presence of the CMY-2 β-lactamase, the total protein extraction corresponding to CMY-2 producing *E. coli* 9238-3013 and the non-CMY-2 producing *E. coli* T1 strains were initially dried under vacuum (speed-vac) 70 min to evaporate the organic solvent. Next, a volume containing 20 μg of protein was subjected to disulfide bond reduction with 20 mM dithiothreitol for 45 min at 56 °C, and alkylation with 20 mM iodoacetamide for 45 min at room temperature in the dark. Both samples were dissolved in 50 mM NH_4HCO_3 buffer, pH 8. Finally, trypsin proteolytic digestion was performed overnight. The samples were analyzed by nanoHPLC (EASY-Spray Accucore, Thermo scientific, West Palm Beach, FL, USA) coupled to a mass spectrometer with Orbitrap technology (Q-Exactive, Thermo Scientific, West Palm Beach, FL, USA), enabling both separation and identification of peptides. Ionization of samples was made by electrospray (EASY-SPRAY, Thermo Scientific, West Palm Beach, FL, USA) and data analysis was performed by the Proteome Discoverer software version 1.4, Thermo Scientific. Coverage percentages were calculated based on the number of identified peptides/total number of peptides.

2.6 Direct detection of CMY-2 from blood culture broths using MALDI-TOF MS

An experimentally test was carried out on blood cultures in order to evaluate the present methodology directly on these samples. Three blood culture bottles (BACTEC® Plus Aerobic bottles) were inoculated with 2 ml of human blood mixed with different bacterial suspensions (approximately 10^4 CFU/ml): *E. coli* 9238-3013 and *E. coli* 47688 (both CMY-2 producers) and *E. coli* T1 (CTX-M-15 producer, as negative control). Blood cultures were incubated at 37 °C for 18 h. To remove blood cells, 1.4 ml of the positive cultures were centrifuged at 200 x *g* for 5 min. The supernatant was then centrifuged at 14,170 x *g* for 1 min at room temperature (Rodríguez-Sánchez et al., 2014). Further, each pellet was subjected to the same protein extraction methodology described above. All samples were tested in duplicate.

3 Result and discussion

Initial experiments were focused on obtaining characteristic MALDI-TOF-MS spectra, especially in the range between m/z 35,000 – 45,000 Da. Thus, CMY-2 producing *E. coli* HBC1a1 (transconjugant) and *E. coli* HB101 (recipient) were processed, and a characteristic peak around 39,800 Da (slightly differing from the expected value for the mature CMY-2 enzyme, 39,854 Da) was observed only in the transconjugant strain (Figure 1a). In addition, *E. coli* DH5α harboring the recombinant plasmid pBC-SK- CMY-2 (called pBL-II-CMY-2) showed this specific peak when was compared with the isogenic strain (*E. coli* DH5αpBC-SK) (Figure 1b). These CMY-2-producing strains were used as positive control when the different wild-type isolates were challenged.

Subsequently, all clinical enterobacterial isolates (n = 63) included in the study were processed under the same conditions by MALDI-TOF MS. Comparison among mass spectral profile of different *E. coli* clinical strains showed that two groups of isolates could be differentiated after peak analysis in the m/z range above mentioned. A single distinctive peak with different intensities, at approximately m/z 39,800 Da was found in all CMY-2 producing *E. coli* strains) and consistently absent in the CMY-2 negative strains included (Figure 1c). The presence of this distinctive peak was also dobserved in CMY-2 producing isolates of other *Enterobacterales* members (Table 1 and Figure 2). Analysis of replicate cultures of all isolates gave simple and consistent mass spectral profiles within this range.

Statistical analysis was performed using the full raw spectra (17,000 to 50,000 Da) and the "Peak Statistic Calculation" (ClinProTools) which included the 39,800 Da-peak corresponding to mature CMY-2 protein. Statistical results showed a *p*-value < 0.000001 (by both PAD and PWKW tests) which confirmed a significant difference for the selected peak (Stephens, 1974). Consistently, the area under the curve (AUC) of the ROC curve for this specific peak was 1.0 (100 % values for sensitivity and specificity), indicating a perfect test and a high discriminative power.

In order to assess if the CMY-2 peak was altered in *E. coli* depending on ampicillin induction, about one half (17/29) CMY-2 producers were compared after growth in medium with or without ampicillin (100 mg/L). Results did not show any difference in intensity or position (*m/z*) of this distinctive peak (39,800 Da) in the spectra (Figure 1d), which is consistent with the lack of AmpR-regulator in this mechanism.

The presence of CMY-2 in *E. coli* 9238-3013, positive in the MALDI-TOF MS assay, was reliably confirmed by protein identification by NanoHPLC coupled to a mass spectrometer with Orbitrap technology which revealed two CMY-2 compatible peptides (Table 2). Consistently, these two peptides were not detected in the non-CMY-2 producing culture (*E. coli* T1).

Finally, the CMY-2 β-lactamase could be directly detected from blood culture bottles.. In good agreement with our previous results, the distinctive peak of approximately 39,800 Da, corresponding to CMY-2, was observed in both spectra of CMY-2-producing *E. coli*, but not in the CTX-M-15 producer.

In this study, we demonstrated that MALDI-TOF MS has the potential to detect, in a quick, easy and inexpensive procedure, the most clinically relevant acquired AmpC β-lactamase, the CMY-2-enzyme, both on *E. coli* plates, and even in other *Enterobacterales*, however the incidence of this enzyme in other species than *E. coli* is negligible. Even if still preliminary, this approach may be useful directly on positive blood cultures bottles.

Once the technology is in place, MALDI-TOF MS applications are fast and low-cost techniques. MALDI-TOF MS approaches are faster than any phenotypic or genotypic procedure currently used in the clinical laboratory for resistance marker detection, as disk diffusion method and PCR plus sequencing. Compared with previous MALDI-TOF MS procedures developed for detection of CMY-2 producing *E. coli* (Papagiannitsis et al., 2014), the present one employs a single solvent extraction (formic acid - isopropyl alcohol - water) step without any incubation period consuming less than 1-h turnaround time. Moreover, this can be run while processing samples for microbial identification and thus several samples may be run almost simultaneously without a

mandatory end point time. Furthermore, the time required is substantially lower than that needed for hydrolysis products detection as well as less labour-consuming (Mirande et al., 2015; Papagiannitsis et al., 2014). The procedure presented in this study, does not require additional reagents to those available and used routinely for microorganism identification by MALDI-TOF MS, except for isopropyl alcohol and sinapinic acid.

This simple approach requires an additional acquisition method optimized for the mass range from 17,000 – 50,000 Da and also, an extra independent sample preparation is necessary to assess the resistance marker detection. Nevertheless, this improvement is an important step towards routine application.

Our results may constitute the basis for further research to detect in a rapid and reliable way other proteins, under similar conditions. Overall, if the method can be extended to other β-lactamases, or even other resistance markers, may prove a significant improvement in laboratory abilities for their detection with extensive clinical implications, and we strongly believe that appropriate validations will definitely lead to establishing a MALDI-TOF supplementary database for future applications in diagnostic laboratories and reference centers.

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Transparency declarations

None to declare

Conflicts of interest

The authors declare that they have no conflicts of interest

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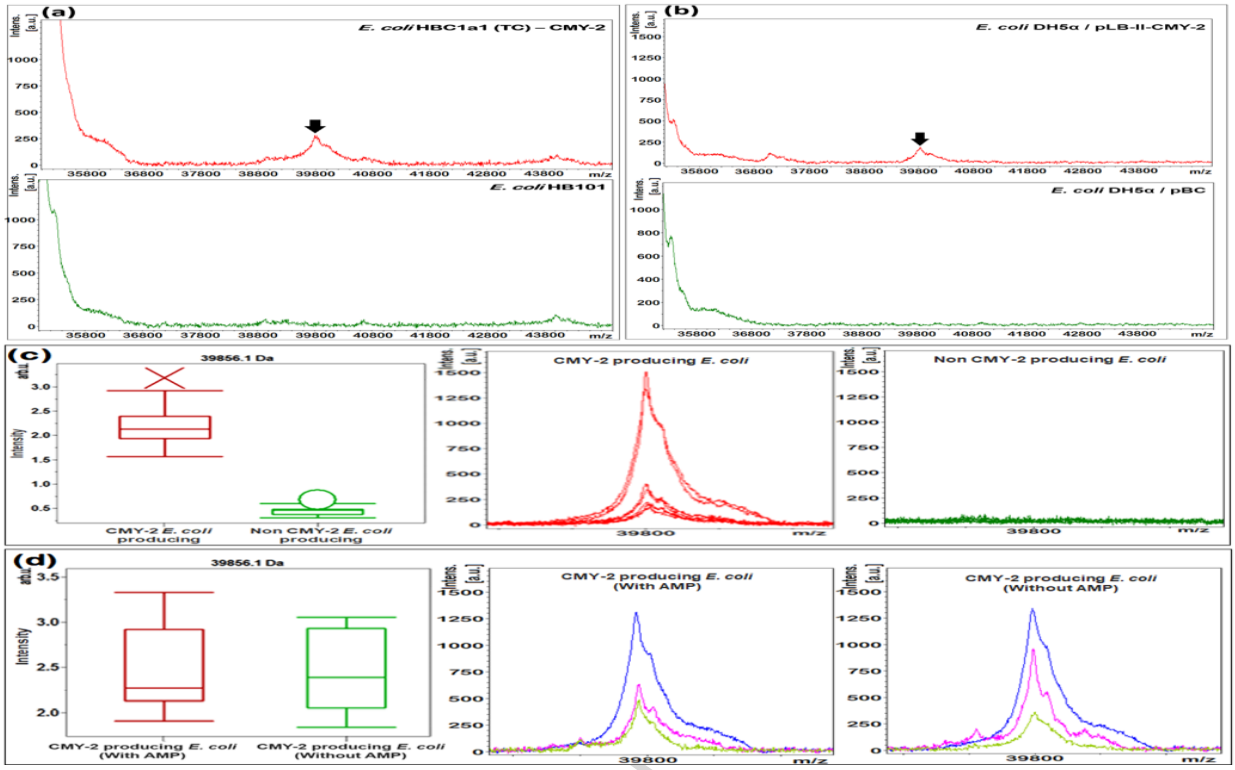

Figure 1.Peaks of MALDI-TOF MS of the CMY-2 and non-CMY-2 producing *E. coli* using a mass range from 35,000 to 45,000 Da. **(a)** Peak detection of ~39,800 Da in the *E. coli* transconjugant strain (HBC1a1) is indicated with arrow on the red line plot; absence of the 39,800-m/z peak in the recipient strain (HB101) is observed on the green line plot. **(b)** Peak detection of ~39,800 Da in the *E. coli* harboring the recombinant plasmid with CMY-2 (*E. coli*DH5α/pBC-SK-CMY-2) is indicated with arrow on the red line plot; absence of the 39,800-m/z peak in the isogenic strain (*E. coli* DH5α/pBC-SK) is observed on the green line plot.**(c)** Box plots and spectra of the 39,800-m/z peak corresponding to CMY-2 detection in clinical isolates of CMY-2 producer are shown with red color. Spectra of non-CMY-2-producing isolates (negative controls) are shown with green color. **(d)** Comparison of spectra using ampicillin (100 µg/ml) as inductor. Box plots and spectra of three different CMY-2 positive *E. coli* grown in presence and in absence of ampicillin (AMP). A specific color (blue, magenta and green) represents the same clinical isolate in both conditions. *x* and *y* axes show *m/z* values and intensity (in arbitrary units), respectively.

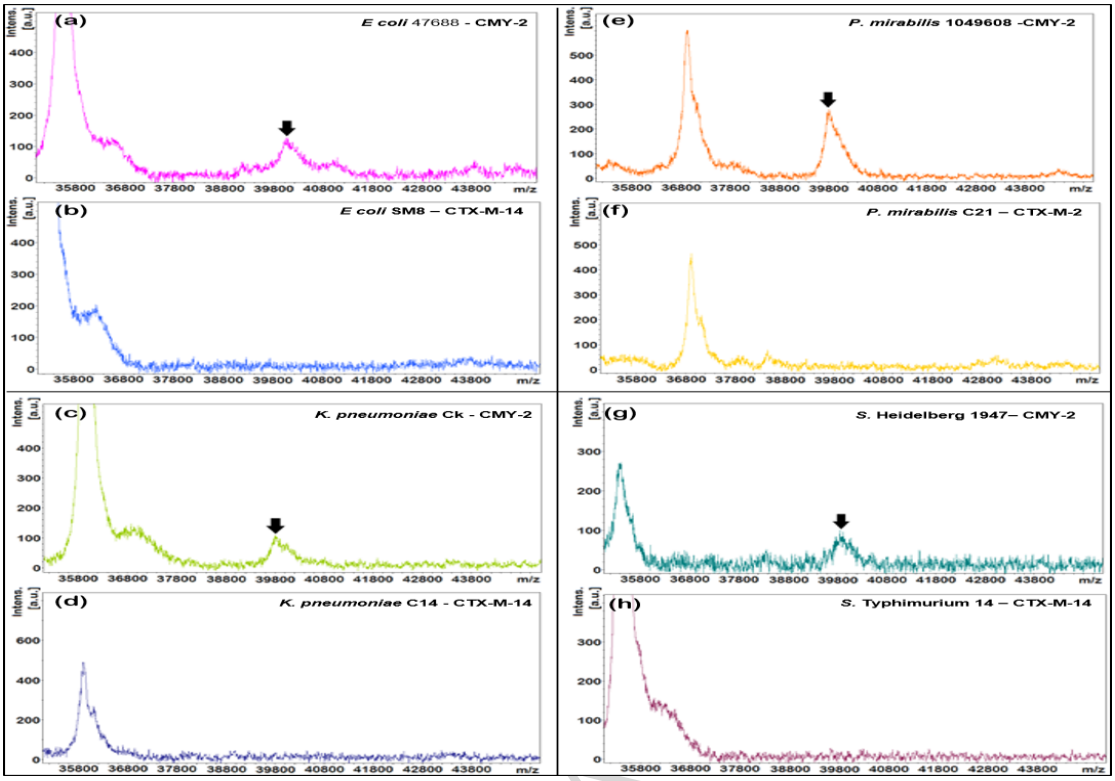

Figure 2. Comparison of peak profiles (m/z) generated by MALDI-TOF MS in the molecular mass spectra from 35,000 to 45,000 Da. Mass peaks corresponding to CMY-2 β-lactamases (~39,800 Da) are indicated with arrows with solid lines. This peak was detected in representative clinical isolates of CMY-2-producing *E. coli* (a), *K. pneumoniae* (c), *P. mirabilis* (e) and *S. enterica* (g), and was consistently absent in non-CMY-2-producing isolates of *E. coli* (b), *K. pneumoniae* (d), *P. mirabilis* (f) and *S. enterica* (h).

Table 1. Characterization of CMY-2-producing isolates by MALDI-TOF MS analysis

Strain	Isolate Origin	Resistance marker	Peak at m/z ~39,800
E. coli VCMY-3	Pet/Dog	bla_{CMY-2}	+
E. coli VCMY-25	Pet/Dog	bla_{CMY-2}	+
E. coli VCMY-27	Pet/Dog	bla_{CMY-2}	+
E. coli VCMY-35	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-51	Pet/Dog	bla_{CMY-2}	+
E. coli VCMY-89	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-103	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-104	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-105	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-106	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-107	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-108	Pet/Cat	bla_{CMY-2}	+
E. coli VCMY-110	Pet/Cat	bla_{CMY-2}	+
E. coli 4820	Human / Nosocomial	bla_{CMY-2}	+
E. coli 4598	Human / Nosocomial	bla_{CMY-2}	+
E. coli 47688	Human / Community	bla_{CMY-2}	+
E. coli 47914	Human / Community	bla_{CMY-2}	+
E. coli 9233-3045	Human / Community	bla_{CMY-2}	+
E. coli 9234-26	Human / Nosocomial	bla_{CMY-2}	+
E. coli 9316-3009	Human / Community	bla_{CMY-2}	+
E. coli 9238-3013	Human / Community	bla_{CMY-2}	+
E. coli 9272-3113	Human / Community	bla_{CMY-2}	+
E. coli 9288-3031	Human / Community	bla_{CMY-2}	+
E. coli 3945440-1	Human / Nosocomial	bla_{CMY-2}	+
E. coli 3937856	Human / Nosocomial	bla_{CMY-2}	+
E. coli MI78/09	Human / Nosocomial	bla_{CMY-2}	+
E. coli 3335	Human / Nosocomial	bla_{CMY-2}	+
E. coli ELA069956	Human / Community	bla_{CMY-2}	+
E. coli CLB020365	Human / Community	bla_{CMY-2}	+

E. coli SM5	Human / Nosocomial	$bla_{\text{CTX-M-15}}$	-
E. coli SM7	Human / Nosocomial	$bla_{\text{CTX-M-14}}$	-
E. coli SM8	Human / Nosocomial	$bla_{\text{CTX-M-14}}$	-
E. coli L4	Human / Nosocomial	$bla_{\text{CTX-M-15}}$	-
E. coli T1	Human / Nosocomial	$bla_{\text{CTX-M-15}}$	-
E. coli T3	Human / Nosocomial	$bla_{\text{CTX-M-15}}$	-
E. coli M1	Human / Nosocomial	$bla_{\text{CTX-M-15}}$	-
E. coli SM3	Human / Nosocomial	$bla_{\text{CTX-M-2}}$	-
E. coli VTEM-37	Pet/Dog	$bla_{\text{TEM-1}}$	-
E. coli VTEM-38	Pet/Dog	$bla_{\text{TEM-1}}$	-
E. coli VTEM-82	Pet/Dog	$bla_{\text{TEM-1}}$	-
E. coli VTEM-86	Pet/Dog	$bla_{\text{TEM-1}}$	-
E. coli VTEM-93	Pet/Dog	$bla_{\text{TEM-1}}$	-
E. coli SA15	Human / Community	$bla_{\text{TEM-1}}$	-
E. coli SA18	Human / Community	$bla_{\text{TEM-1}}$	-
E. coli SA78	Human / Community	$bla_{\text{TEM-1}}$	-
E. coli VS6	Pet/Dog	ND	-
E. coli VS15	Pet/Dog	ND	-
E. coli CEM4	Human / Community	ND	-
E. coli CEM9	Human / Community	ND	-
E coli IACA17	Human / Community	ND	-
E. coli HBC1a1 (TC)	Laboratory Strain	*rps*L/ $bla_{\text{CMY-2}}$	+
E. coli HB101	Laboratory Strain	*rps*L	-
E coli DH5α	Laboratory Strain	*gyr*A96	-
E. coli DH5α + pBC-SK	Laboratory Strain	*cat*A1	-
*E. coli*DH5α + pBC-SK- CMY-2(pBL-II-CMY-2)	Laboratory Strain	*cat*A1/$bla_{\text{CMY-2}}$	+
E. coli ATCC 25922	Laboratory Strain	ND	-
E. coli ATCC35218	Laboratory Strain	$bla_{\text{TEM-1}}$	-
K. pneumonia Ck	Human / Nosocomial	$bla_{\text{CMY-2}}$	+
K. pneumonia C14	Human / Nosocomial	$bla_{\text{CTX-M-2}}$	-
K. pneumonia C10	Human / Nosocomial	$bla_{\text{CTX-M-2}}$	-
K. pneumonia 372952	Human /	$bla_{\text{KPC-2}}$ /$bla_{\text{CTX-}}$	-

	Nosocomial	M-2	
P. mirabilis 1049608	Human / Nosocomial	bla_{CMY-2}	+
P. mirabilis C13	Human/ Nosocomial	$bla_{CTX-M-2}$	-
P. mirabilis C21	Human/ Nosocomial	$bla_{CTX-M-2}$	-
S. Heidelberg 1947	Human/ Community	bla_{CMY-2}	+
S. Heidelberg 2089	Human/ Community	bla_{CMY-2}	+
S. Enteritidis S08	Human / Community	ND	-
S. Infantis S11	Human / Nosocomial	bla_{TEM-1}	-
S. Infantis S21	Human / Nosocomial	$bla_{CTX-M-2}$	-
S. Typhimurium 14	Human/ Community	$bla_{CTX-M-14}$	-
TC: Transconjugant strain, ND: Non-detectable β-lactamases, +: peak observed, -: peak not observed			

Table 2. Peptides detected by NanoHPLC coupled to a mass spectrometer with Orbitrap technology

Sample	**Resistance Associated**	**Observed Peptide**	**Theoretical molecular weightDa**	**Expected value Da $[M+H]^+$**	**Amino acid position**
E. coli 9238-3013	CMY-2	AAKTEQQIADIVNRTITP	1969.23	1969,07	21-38
		AAKTEQQIADIV	1286.45	1286,69	21-32
CMY-2 amino acid sequence from *E. coli* (GenBank accession no. AB212086) was used as a reference for the alignment of β-lactamase peptides					

Highlights

- The CMY-2 β-lactamase was directly detected by MALDI-TOF MS.
- A new, fast and low-cost protocol with a less time-consuming manner was developed.
- A distinctive peak around 39,800 Da was found in all CMY-2 producing strains.
- A perfect test and a high discriminative power were observed.
- Direct CMY-2 detection on positive blood cultures bottles was achieved.