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Proceeding Paper

Rapid Detection of Foodborne ESBL-Producing Enterobacteriaceae Using MALDI-TOF Mass Spectrometry †

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Abstract: The proliferation of enterobacteria that produce extended spectrum β-lactamases (ESBL) has become an important public health concern. The objective of this work was to optimize a phenotypic method for straightforward and rapid ESBL detection in strains obtained from food environments, employing MALDI-TOF mass spectrometry. A collection of 162 enterobacterial isolates, including ESBL producers, AmpC producers, ESBL-AmpC coproducers and sensitive strains was analyzed using a quick method based on cefotaxime hydrolysis. Statistical analysis showed that, although the presence of constitutive AmpC hyperproducers could reduce its specificity, this MALDI-TOF assay is an excellent tool for quickly classifying ESBL-producing strains of foodborne Enterobacteriaceae.

Keywords: antibiotic resistance detection; MALDI-TOF; β-lactamases; foodborne microorganisms

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1. Introduction

The increasing prevalence of microorganisms producing extended-spectrum (ESBLs) and AmpC β-lactamases poses a critical global challenge in combating antibiotic resistance [1].

Classical approaches for detection of antimicrobial resistance include disc diffusion and microdilution methodologies whose protocols involve isolation and incubation with antibiotic steps, which can delay the acquisition of a result up to three days and can be time consuming. In clinical settings, obtaining rapid results can be of critical importance in order to make decisions on the treatment of bacterial infections that can be life threatening. Development of innovative techniques with this objective is therefore essential.

Mass spectrometry for the detection of β-lactamase producing bacteria has been successfully implemented in clinical laboratories [2] but its application on surveillance and monitoring efforts has not been optimized yet despite its potential to facilitate acquiring data on a bigger scale.

Our objective was to optimize a method for straightforward and rapid detection of β-lactamase production in strains obtained from environmental and clinical settings employing MALDI-ToF mass spectrometry.

2. Material & Methods

2.1. Strain Selection

Strains were classified based in the outcome of MAST-D72C (MAST group, UK) and/or microdilution testing in five categories: ESBL positive, constitutive AmpC, inducible AmpC, ESBL + AmpC coproducers and non-beta-lactamase producing strains. Table 1 provides detailed information about the selected strains, which were obtained from various sources, including fresh vegetables, fresh meat, dairy products, wastewater, clinical and collection strains.

Table 1. Strain selection of the study. [1]: constitutive AmpC producer, [2]: inducible AmpC producer; [3]: ESBL + cAmpC coproducer, [4]: non-beta-lactamase producing strains.

Species	ESBL	cAmpC [1]	iAmpC[2]	Coproducer [3]	NBLP [4]
Escherichia coli	51	0	0	1	35
Escherichia fergusonii	2	0	0	0	0
Enterobacter spp.	0	9	4	1	0
Citrobacter spp.	0	11	10	0	0
Hafnia alveii	0	6	0	0	0
Lelliotia amnigena	0	2	0	0	0
Morganella morganii	0	0	1	0	0
Klebsiella pneumoniae	1	0	0	0	0
Klebsiella oxytoca	1	0	0	1	0
Rahnella aquatilis	10	0	0	0	0
Serratia fonticola	15	0	0	0	0
Scandinavium spp.	0	0	0	1	0
Total	**80**	**28**	**15**	**4**	**35**

2.2. Antibiotic Hydrolysis Assay and Spectrum Acquisition

Test strains were grown on Mueller Hinton Agar (OXOID, UK) for 18–24 h at 37 °C. A 1 μL loopful of bacterial biomass was resuspended in a 0.5 μg/mL cefotaxime solution in antibiotic reaction buffer (10 mM NH_4CO_3/10 ug/mL $ZnCl_2$/0.005% SDS) and incubated for 1 h at 37 °C with continuous agitation. After the incubation period, bacterial suspensions were centrifuged at 14,000 rpm for 3 min. Equal parts of the obtained supernatant were mixed with HCCA matrix (Thermofisher, UK) dissolved in 50 HCN/49.9% H_2O/0.1% TFA solution. One μL of this mixture was laid on MALDI Target and allowed to dry avoiding direct light exposure. Spectra were acquired on MALDI-TOF MS microflex (Bruker) between a 0–1000 Da range. MBT-star-ACS solution was employed for calibration using 243.1, 403.3, 573.3 and 757.3 m/z peaks.

2.3. Spectra Data Processing

Acquired spectra were analyzed using FlexAnalysis software (Bruker). 370–414 ± 0.5 and 456–478 ± 0.5 Da peaks were selected as representative of the hydrolyzed and non-hydrolyzed forms of cefotaxime, respectively, in accordance with SEIMC indications [3]. Spectra were selected for processing if the signal to noise ratio of at least one of the pairs of peaks associated with hydrolyzed or non-hydrolyzed forms of the antibiotic was above 5. Intensity of filtered peaks was used for calculating logRQ parameter [4] using the following equation:

$$logRQ = \left(\frac{i370 + i414}{i456 + i478}\right)$$

Average logRQ from three replicates were normalized between 0–1 values using an excel datasheet.

$$Normalized\ logRQ = \frac{x\ logRQ - log\ RQ\ min}{logRQ\ max - logRQ\ min}$$

2.4. Statistical Analysis

Hydrolysis peaks were transformed to logRQ and normalized in a datasheet (Microsoft Excel). All values were statistically analyzed for obtaining means and standard deviation. The Levene statistic was performed for testing the homogeneity of group variances. Robust tests of equality of means were performed (Welch and Brown-Forsythe). The effect of types (strains) on the investigated variable (normalized logRQ) was carried out with the Kruskal-Wallis one-way analysis of variance (ANOVA) and post hoc Tamhane's test (unequal variances). The IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA) program was used for data analysis.

3. Results & Discussion

Figure 1 showed a non-ESBL producer exhibiting peaks at 456 and 458 *m*/*z*, corresponding to the non-hydrolyzed form of cefotaxime. An ESBL-producing strain that hydrolysed cefotaxime consequently produced peaks at 370 and 414 *m*/*z*, while the peaks at 456 and 458 *m*/*z* diminished.

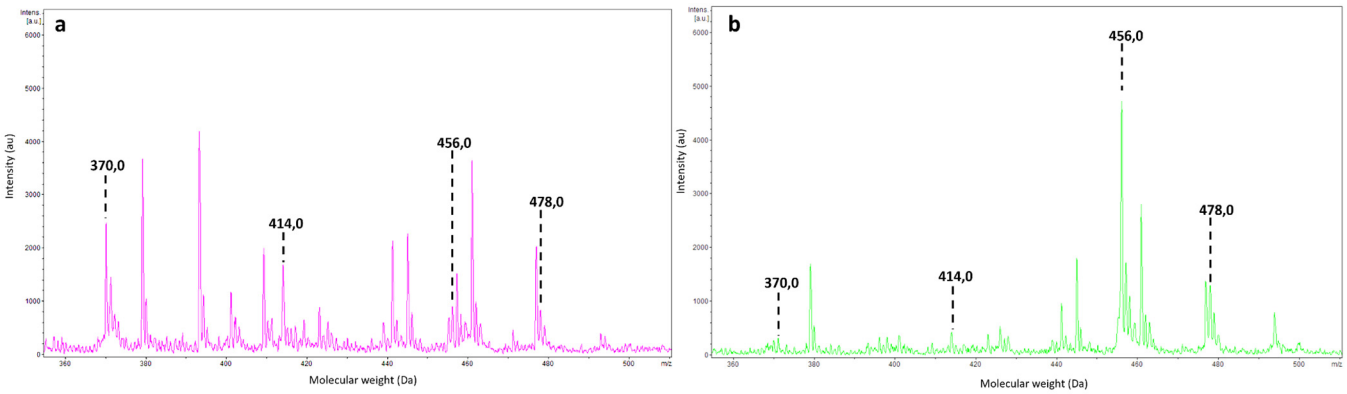

Figure 1. MALDI-TOF spectra of the hydrolyzed form of cefotaxime (**a**) obtained from an ESBL producing strain and the corresponding non-hydrolyzed form (**b**) from a non-ESBL producing strain.

The MALDI-TOF-based ESBL assay for *Enterobacteriaceae* strains isolated from food and their production environment yielded a global sensitivity of 100% and a specificity of 94.8% (confidence interval: 87.2–98.4%), considering all the different types of tested strains in this study (Figure 2 and Table 2). These parameters were obtained from the ROC curve for an optimal cutoff point where sensitivity was maximized. Our results on food strains are comparable to those reported by Oviaño et al. [5] who validated a similar procedure for the classification of ESBL and AmpC producers in positive blood cultures (clinical isolates). They found that the use of cefotaxime resulted in higher sensitivity. Our approach used a more easily understandable normalization procedure (logRQ values from 0 to 1) which would facilitate its application in other laboratories due to its more intuitive interpretation.

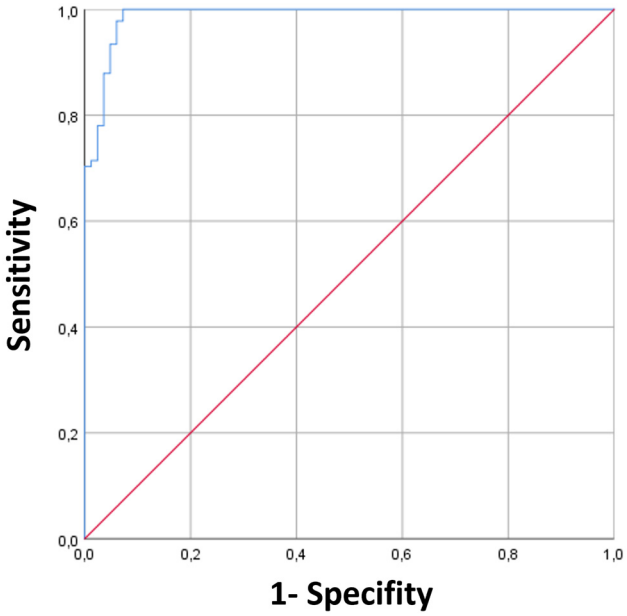

Figure 2. ROC curve obtained analyzing the sensitivity and specificity of the method for detecting ESBL producing isolates.

Table 2. Threshold values (normalized logRQ) and their respective sensitivity and specificity for the ESBL producing isolates detection method.

Threshold Value	Sensitivity (%)	Specificity (%)	AUC	Asintotic Significance
0.000	100	0.0		
0.100	100	42.7		
0.210	**100**	**94.8**	0.990	0.000
0.231	97.5	95.8		
0.331	80.0	96.9		

All the ESBL producers were accurately identified as positive with a normalized logRQ threshold > 0.210, but there were five *Enterobacteriaceae* strains that hyperproduced AmpC included (false positives). It's important to emphasize that the non-clinical strains co-producing AmpC and ESBL enzymes were detected as negative by the assessed method (Figure 3).

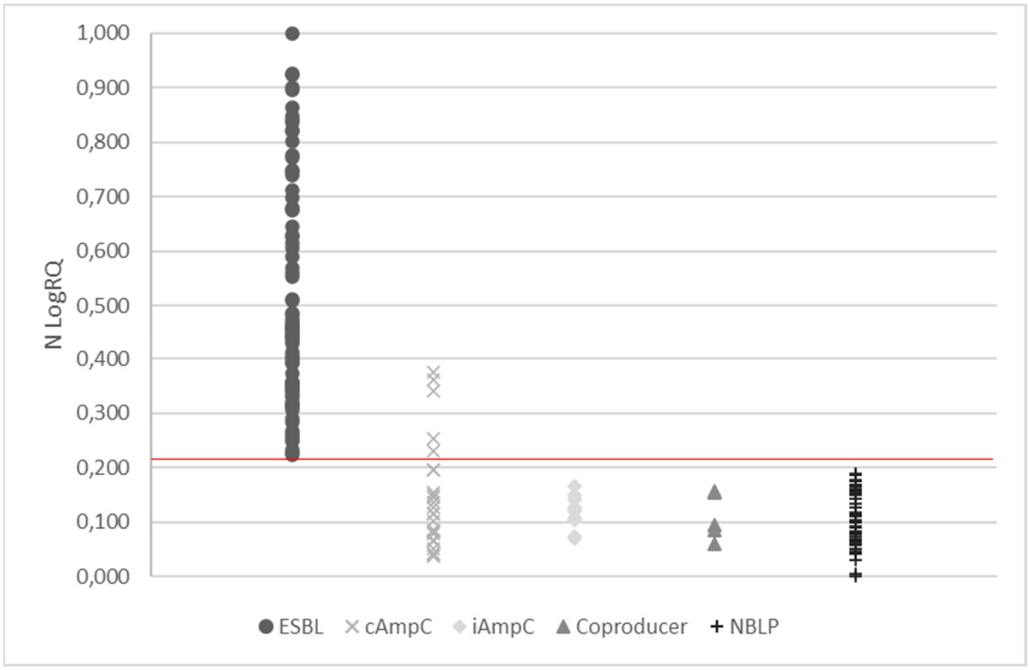

Figure 3. Distribution of normalized LogRQ values across the different categories of strains using the MALDI-ToF approach (ESBL, ESBL-producing strain; cAmpC, constitutive-AmpC producing strain; iAmpC, inductive-AmpC producing strain; Coproducer, ESBL and AmpC co-producing strain; NBLP, non-B-lactamase producing strain.

Hydrolysis products of cefotaxime generated by AmpC beta-lactamases are likely different from those generated by ESBL [6]. As a result, they would exhibit different masses. Efforts to identify and distinguish the peaks associated with these metabolites must be conducted in order to accurately classify AmpC-producing strains, particularly those hyperproducers, and to increase the specificity of ESBL detection using this methodology.

This study also sheds new light on how constitutive AmpC producers, which we have associated with AmpC-hyperproducing *Enterobacteriaceae* strains, can be misidentified as ESBL producers. This fact contributes significantly to the lack of total specificity (Figure 3, Table 2). This unspecificity has been also observed in other similar rapid MALDI approaches which do not differentiate the phenotypic expression of AmpC in their validations [5]. Further studies are required to develop similar rapid methods to discriminate strains that exhibit the cAmpC phenotype, especially the hyperproducers that the assessed MALDI-ToF approach categorized as "ESBL", as well as those strains that co-produced AmpC and ESBL enzymes.

Environmental ESBL strains are generally more susceptible to antibiotics, and food-related strains are no exception to this assumption. This study focused on the detection of food-related ESBL strains, establishing new thresholds for their detection. Our results indicate that, despite the presence of constitutive AmpC hyperproducers could potentially reduce its specificity, this MALDI-TOF assay, based on the cefotaxime hydrolysis, demonstrates excellent performance in quickly classifying ESBL-producing strains of *Enterobacteriaceae* isolated from non-clinical environments, including foods and production farms.

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References

1. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention: Antibiotic Resistance Threats in the United States. 2019. Available online: https://www.cdc.gov/drugresistance/pdf/threatsreport/2019-ar-threats-report-508.pdf (accessed on 26 October 2023).
2. Oviaño, M.; Gómara, M.; Barba, M.J.; Revillo, M.J.; Barbeyto, L.P.; Bou, G. Towards the early detection of β-lactamase-producing Enterobacteriaceae by MALDI-TOF MS analysis. *J. Antimicrob. Chemother*. **2017**, *72*, 2259–2262. https://doi.org/10.1093/jac/dkx127.
3. Cercenado Mansilla, E.; Cantón Moreno, R.; Oviaño García, M.; Rodríguez Sánchez, B.; Caballero Pérez, J.D.; Muñoz Bellido, J.L. Aplicaciones de la Espectrometría de Masas MALDI-TOF en Microbiología Clínica en Procedimientos en Microbiología Clínica Recomendaciones de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. 2019. Available online: https://seimc.org/contenidos/documentoscientificos/procedimientosmicrobiologia/seimc-procedimientomicrobiologia65.pdf (accessed on 5 September 2023).
4. Jung, J.S.; Popp, C.; Sparbier, K.; Lange, C.; Kostrzewa, M.; Schubert, S. Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Detection of β-Lactam Resistance in Enterobacteriaceae Derived from Blood Cultures. *J. Clin. Microbiol.* **2014**, *52*, 924–930. https://doi.org/10.1128/JCM.02691-13.
5. Oviaño, M.; Fernández, B.; Fernández, A.; Barba, M.J.; Mouriño, C.; Bou, G. Rapid detection of enterobacteriaceae producing extended spectrum beta-lactamases directly from positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Clin. Microbiol. Infect*. **2014**, *20*, 1146–57. https://doi.org/10.1111/1469-0691.12729.
6. Li, C.; Ding, S.; Huang, Y.; Wang, Z.; Shen, J.; Ling, H.; Xu, Y. Detection of AmpC β-lactamase-producing Gram-negative bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Hosp. Infect.* **2018**, *99*, 200–207. https://doi.org/10.1016/j.jhin.2017.11.010.