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

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Sir,

The spread of *bla*$_{KPC}$ genes responsible for the production of KPC carbapenemases among the family of Enterobacteriaceae (in particular *Klebsiella pneumoniae* species) is reaching alarming rates in America, Europe and parts of Asia.[1] So far, nine different *bla*$_{KPC}$ genes (i.e. *bla*$_{KPC-2}$ to *bla*$_{KPC-10}$) encoding enzymes with very similar kinetic characteristics have been reported.[1,2]

Production of KPC carbapenemases confers resistance to all classes of β-lactams including those combined with standard β-lactamase inhibitors. In addition, isolates producing this enzyme are frequently co-resistant to quinolones, aminoglycosides and occasionally to colistin and fosfomycin.[1,3,4] As a result, the rapid identification of *bla*$_{KPC}$-possessing Enterobacteriaceae has serious implications for the outcome of infected patients. However, detection of *bla*$_{KPC}$-possessing isolates presents a significant challenge for clinical laboratories.[5,6] Therefore, a rapid and accurate system to identify these life-threatening pathogens would be welcome.

The PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS) technology is a promising genotyping system that possesses high multiplexing capacity and can be used for detecting different genes (e.g. resistance traits and/or virulence factors) present in a single strain. This system can also detect single nucleotide polymorphisms including mutations corresponding to changes in existing amino acids. Primers designed for PCR/ESI-MS yield amplicons that are analytically characterized by high-performance MS and base composition analysis, which is expressed as the relative proportions of A, C, G and T.[7] Previously, we demonstrated that PCR/ESI-MS could accurately detect mutations in *gyrA* and *parC* genes of quinolone-resistant *Acinetobacter baumannii* isolates.[8] In the present work we evaluated the performance of the T5000^{TM} Biosensor System (Ibis Biosciences Inc., Carlsbad, CA, USA), a PCR/ESI-MS platform,[7] for the detection and identification of *bla*$_{KPC}$ genes among Enterobacteriaceae.

One hundred and ten previously characterized Enterobacteriaceae isolates (82 *K. pneumoniae*, 27 *Escherichia coli* and 1 *Enterobacter cloacae*) were used.[3,4,9,10] In particular, there were 69 clinical isolates possessing a *bla*$_{KPC}$ gene (35 *bla*$_{KPC-2}$ and 34 *bla*$_{KPC-3}$)[3,4,9] and 5 *E. coli* DH10B (Invitrogen Corporation, Carlsbad, CA, USA) control strains constructed in our laboratory carrying *bla*$_{KPC-4}$, *bla*$_{KPC-5}$, *bla*$_{KPC-6}$, *bla*$_{KPC-7}$ or *bla*$_{KPC-8}$ in the pBC SK(+) vector. The remaining *bla*$_{KPC}$-negative strains (17 *K. pneumoniae* and 17 *E. coli* isolates) were multidrug-resistant strains that have been described previously.[10] ATCC control strains *E. coli* 25922 and *K. pneumoniae* 700603 were also used.

DNA was obtained by suspending two or three colonies of each test isolate grown on blood-agar plates in 500 μL of nuclease-free water (USB, Cleveland, OH, USA) and heating at 90°C for 10 min using a dry bath incubator (Fisher Scientific, Pittsburgh, PA, USA). Samples were spun at 10000 rpm for 10 min and the resulting supernatant was diluted 1:100 with nuclease-free water. Aliquots of 10 μL were directly added to each of the 96 wells of the KPC Resistance Assay Kit plate (Ibis Biosciences Inc.) to a total of 24 samples per plate. Additional reagents were not necessary because each well contained 40 μL of a pre-loaded mixture consisting of buffer, dNTPs, enzyme and primers. In its current format this research kit uses primers designed to detect all known genes encoding KPC variants and to differentiate *bla*$_{KPC-3}$ from *bla*$_{KPC-4}$/*bla*$_{KPC-5}$ and other KPC types. A primer pair specific for the valyl-tRNA synthetase (*valS*) housekeeping gene has also been included for species identification of Enterobacteriaceae.[7] The primer pairs used in the kit are reported in Table 1.

Plates were incubated in the Mastercycler pro S Thermal Cycler (Eppendorf, Hauppauge, NY, USA) with the following PCR cycling conditions: 95°C for 10 min; 8 cycles of 95°C for 30 s, 48°C for 30 s and 72°C for 30 s; 37 cycles of 95°C for 15 s, 56°C for 20 s and 72°C for 20 s; 72°C for 2 min; and 99°C for 20 min (total, 2 h and 21 min). Plates were then loaded onto the automated T5000^{TM} Biosensor System for analysis and interpretation of PCR amplicons as previously reported (total, 2 h and 30 min for one plate).[7]

Our results show that the T5000^{TM} System correctly identified all 74 *bla*$_{KPC}$-possessing strains; false-positive results were not observed (sensitivity and specificity of 100%). In particular, all *bla*$_{KPC-2}$- and *bla*$_{KPC-3}$-possessing strains were correctly reported. Notably, we were also able to identify all five KPC-positive *E. coli* DH10B control isolates, but we were 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.

In conclusion, the results of the present study show that the T5000^{TM} System is an accurate tool to detect *bla*$_{KPC}$-possessing Gram-negative isolates. Due to its rapid performance, the PCR/ESI-MS-based platform could be used in the clinical setting to improve the outcome of infected patients and could also be

Table 1. Primers pairs used by the KPC Resistance Assay Kit to obtain the specific bla_{KPC} amplicons analysed by ESI-MS[a]

Primer pair name[b]	4674	4675	4676
forward (5′–3′)	TTGCTGGACACACCCATCCGTTAC	TACACCCGGACGCCTAACAAGGA	TGGAGCTGAACTCCGCCATCC
reverse (5′–3′)	TCTCCGCCACCGTCATGCCTG	TGCCCGTTGACGCCCAATCC	TCCAGTGCAGAGCCCAGTGTCAG
Expected base composition after ESI-MS analysis[c]			
bla_{KPC-2} and all other bla_{KPC} subtypes not listed below	A26 G26 C26 T19	A22 G31 C30 T12	A23 G28 C33 T16
bla_{KPC-3}	A26 G26 C26 T19	**A22 G31 C29 T13**	A23 G28 C33 T16
bla_{KPC-4} and bla_{KPC-5}	**A26 G27 C25 T19**	A22 G31 C30 T12	A23 G28 C33 T16

[a]Species identification of Enterobacteriaceae was obtained with the same kit using primers specific for the *valS* housekeeping gene: 358-forward, 5′-TCGTGGCGGCGTGGTTATCGA-3′; and 358-reverse, 5′-TCGGTACGAACTGGATGTCGCCGTT-3′.
[b]All primer sequences for KPC detection are based on GeneBank ID EU784136.
[c]Results in bold indicate amplicons with different base compositions that identify different KPC variants.

used to perform epidemiological and infection control studies where large collections of isolates need to be rapidly characterized.

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Transparency declarations

R. A. B. has received money for research and accepted speaking invitations from various pharmaceutical companies. None of these poses a conflict of interest with the present work. R. S. and D. J. E. are employees of Ibis Biosciences Inc., a subsidiary of Abbott Molecular Inc. Both R. S. and D. J. E. are shareholders of the company. Other authors: none to declare.

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Elution and antibacterial activity of meropenem from implanted acrylic bone cement

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Sir,

Meropenem has good tissue penetration and broad-spectrum bactericidal activity. Often employed to treat multiresistant