JOURNAL OF CLINICAL MICROBIOLOGY, July 2010, p. 2618–2622 Vol. 48, No. 7
0095-1137/10/$12.00 doi:10.1128/JCM.00568-10
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Evaluation of a Commercial Microarray System for Detection of SHV-, TEM-, CTX-M-, and KPC-Type β-Lactamase Genes in Gram-Negative Isolates▽†

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Received 16 March 2010/Returned for modification 5 May 2010/Accepted 14 May 2010

We evaluated the ability of a commercial microarray system (Check KPC/ESBL; Check-Points Health BV) to detect clinically important class A β-lactamase genes. A total of 106 Gram-negative strains were tested. The following sensitivity and specificity results were recorded, respectively: for bla_{SHV}, 98.8% and 100%; for bla_{TEM}, 100% and 96.4%; and for bla_{CTX-M} and bla_{KPC}, 100% and 100%.

The spread of class A or group 2be extended-spectrum β-lactamases (ESBLs) represents an emerging public-health concern (1, 17). Among the organisms of the *Enterobacteriaceae* family (e.g., *Klebsiella pneumoniae* and *Escherichia coli*), the most frequently detected and clinically important ESBLs belong to the TEM, SHV, and CTX-M families (17). While TEM- and SHV-type ESBLs arise via substitutions in strategically positioned amino acids (e.g., Gly238 and Arg164) from the natural narrow-spectrum TEM-1, TEM-2, or SHV-1 β-lactamase genes, all currently identified CTX-M enzymes demonstrate an ESBL phenotype (7, 14).

The ability to rapidly identify narrow-spectrum β-lactamases (e.g., SHV-11 and TEM-1) and ESBLs (e.g., SHV-5 and SHV-12 or TEM-10) has important clinical implications. Usually, *Enterobacteriaceae* species producing narrow-spectrum enzymes are resistant to penicillins and narrow-spectrum cephalosporins, whereas those producing ESBLs manifest resistance to extended-spectrum oxyimino-cephalosporins and aztreonam (14). Since resistance to quinolones and aminoglycosides is frequently observed among ESBL producers, carbapenems represent one of the therapeutic options of last resort for life-threatening infections due to these organisms (6, 14).

In some geographic areas, the spread of carbapenemases belonging to class A (e.g., KPCs), class B (e.g., VIMs and IMPs), and class D (e.g., OXA-48) has significantly compromised the clinical use of carbapenems, consigning clinicians to the use of "last-line" antimicrobials such as colistin (2, 19). In particular, the KPC β-lactamases (primarily KPC-2 and KPC-3) are the serine carbapenemases that are most widespread in the United States, and strains producing these enzymes are responsible for numerous outbreaks with high mortality rates (3, 9, 13). Although nine KPC-type β-lactamases have been described, their susceptibility profiles are similar, rendering the differentiation of these variants less clinically relevant (13, 22).

Prompt and appropriate antibiotic treatment for infections due to ESBLs- and/or KPC-producing *Enterobacteriaceae* may positively affect the final outcome for infected patients (6, 13). Unfortunately, standard and confirmatory phenotypic tests may fail to identify ESBL- and, more frequently, KPC-producing organisms. For the latter group, the use of the modified Hodge test delays the final report by an additional 24 h (11, 12, 21). Therefore, a rapid and reliable method is needed to perform a quick and accurate analysis of the most important *bla* genes possessed by clinical isolates.

Microarray technologies are promising genotyping systems that possess a high multiplexing capacity and can be used for detecting different β-lactamase genes that are present in a single strain (8, 10, 23). This ability can assist clinicians in directing antimicrobial therapy. In the present work, we evaluated the ability of Check KPC/ESBL (Check-Points Health BV, Wageningen, Netherlands), the first rapid, commercially available, microarray-based diagnostic test system for detection and identification of *bla* genes belonging to the TEM, SHV, CTX-M, and KPC types. This system can detect single nucleotide polymorphisms found in the most important TEM- and SHV-type ESBLs (www.lahey.org/studies), including single mutations corresponding to amino acid positions Val84Ile, Glu104Lys, Arg164Ser/His/Cys, and Gly238Ser in TEMs and Gly238Ser/Ala and/or Glu240Lys in SHVs (7).

A total of 102 *Enterobacteriaceae* and four *Acinetobacter baumannii* isolates possessing different *bla* genes were tested (Table 1; see also Table S1 in the supplemental material). The majority of strains ($n = 61$) had previously been characterized

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† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 26 May 2010.

TABLE 1. Performance of the Check KPC/ESBL microarray assay in identification of β-lactamase genes[a]

Species	bla_{KPC}		bla_{SHV}						bla_{TEM}						bla_{CTX-M}		Isolates with all *bla* genes correctly reported
			ESBL[d]		Non-ESBL		Total		ESBL[d]		Non-ESBL		Total				
	No. of isolates positive by PCR/Seq	Agreement[i] (%)	No. of isolates positive by PCR/Seq	Agreement[i] (%)	No. of isolates positive by PCR/Seq[c]	Agreement[i] (%)	No. of isolates positive by PCR/Seq	Agreement[i] (%)	No. of isolates positive by PCR/Seq	Agreement[i] (%)	No. of isolates positive by PCR/Seq[e]	Agreement[i] (%)	No. of isolates positive by PCR/Seq	Agreement[i] (%)	No. of isolates positive by PCR/Seq	Agreement[i] (%)	Agreement[i] (%)
K. pneumoniae (n = 79)[b]	52	79/79 (100)	41	41/41 (100)	38	31/38 (81.6)	79	72/79 (91.1)	3	3/3 (100)	64	64/64 (100)	67	79/79 (100)	8	79/79 (100)	72/79 (91.1)
E. coli (n = 22)[b]	4	22/22 (100)	3	2/3 (66.7)	2	2/2 (100)	5	21/22 (95.5)	2	2/2 (100)	7	7/7 (100)	9	21/22 (95.5)	9	22/22 (100)	20/22 (90.9)
A. baumannii (n = 4)	0	4/4 (100)	0		0		0	4/4 (100)	2	2/2 (100)			2	4/4 (100)	2	4/4 (100)	4/4 (100)
Enterobacter cloacae (n = 1)	1	1/1 (100)	0		0		0	1/1 (100)	0		1	1/1 (100)	1	1/1 (100)	0	1/1 (100)	1/1 (100)
Total (n = 106)	57	106/106 (100)[f]	44	43/44 (97.7)	40	33/40 (82.5)	84	98/106 (92.5)[g]	7	7/7 (100)	72	72/72 (100)	79	105/106 (99.1)[h]	19	106/106 (100)[f]	97/106 (91.5)

[a] Results represent comparisons of the Check KPC/ESBL microarray assay to PCR amplification followed by DNA sequencing (PCR/Seq).
[b] Includes control isolates.
[c] One *E. coli* isolate containing bla_{SHV-38} was reported as a non-ESBL SHV type; six *K. pneumoniae* isolates containing a $bla_{SHV-non-ESBL}$ gene (i.e., a bla_{SHV-1} or bla_{SHV-11} gene) were reported as representing SHV-ESBL strains; one *K. pneumoniae* isolate containing bla_{SHV-11} was reported as SHV negative (see Table S1 in the supplemental material).
[d] Includes isolates containing bla_{ESBL} (e.g., bla_{SHV-12}) or bla_{ESBL} plus $bla_{non-ESBL}$ genes (e.g., bla_{SHV-12} and bla_{SHV-11}).
[e] One bla_{TEM}-negative *E. coli* isolate was reported as a non-ESBL TEM strain.
[f] Sensitivity, 100%; specificity, 100%; positive predictive value, 100%; negative predictive value, 100%.
[g] Sensitivity, 98.8%; specificity, 100%; positive predictive value, 100%; negative predictive value, 95.7%.
[h] Sensitivity, 100%; specificity, 96.4%; positive predictive value, 98.8%; negative predictive value, 100%.
[i] The value preceding the slash is the number of isolates whose Check KPC/ESBL microarray results agreed with their PCR/Seq results, and the value following the slash is the total number of isolates tested by both assays.

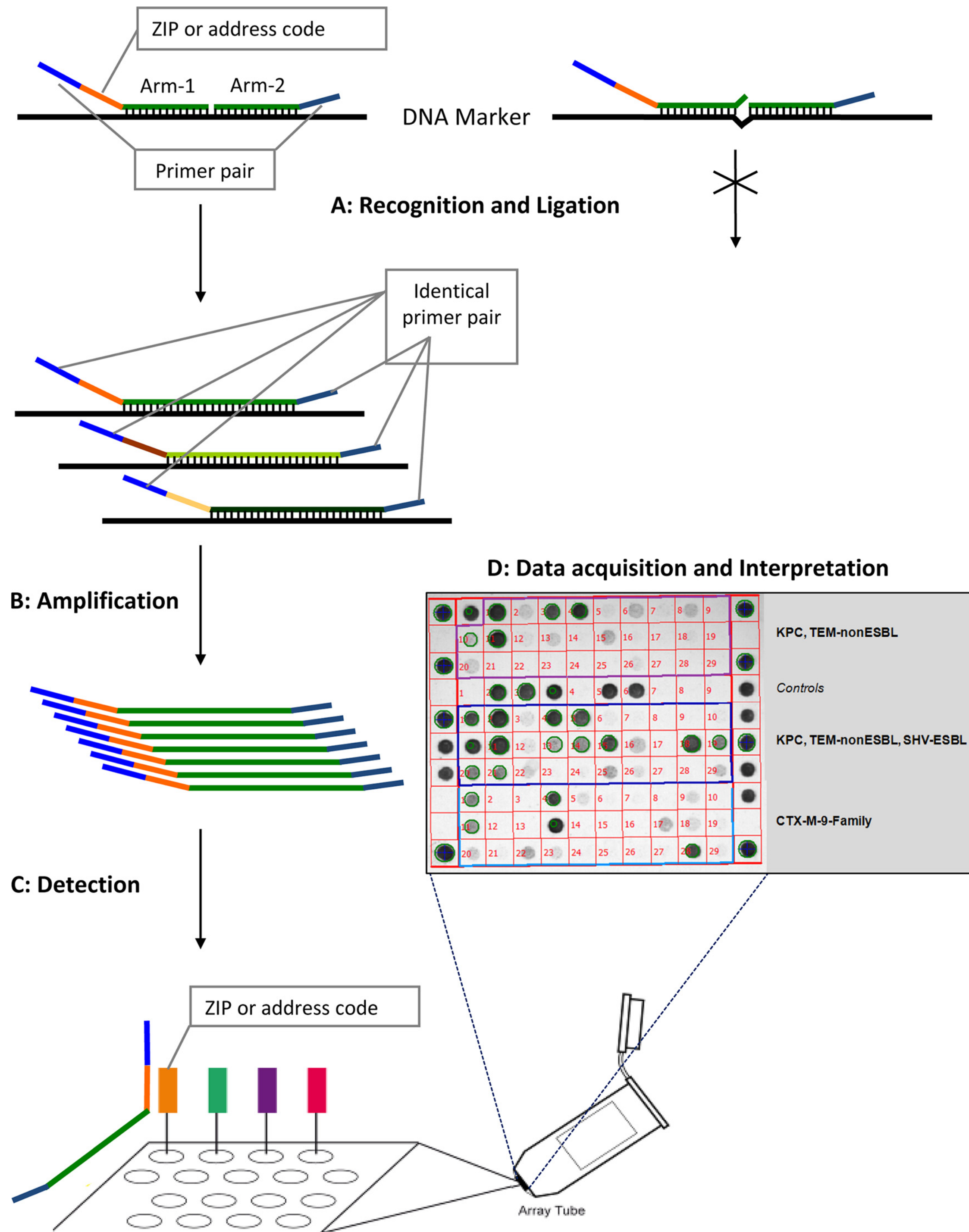

FIG. 1. Schematic flowchart representing the different steps used by the Check-Points KPC/ESBL platform to recognize specific *bla* genes. (Step A) Target DNA recognition and ligation (thermocycling conditions, 95°C for 3 min, 24 cycles of 95°C for 30 s and 65°C for 5 min, and 98°C for 2 min [total, 2.75 h]). Each target-specific probe consists of two oligonucleotide probe arms that are used to detect single nucleotide polymorphisms (SNPs). These two probe arms are connected by the ligase, generating a single probe molecule only when they perfectly match the target sequence. Only connected probe arms produce the labeled amplification products detected in step C. Every target-specific probe is equipped with the same consensus primer pair necessary for step B and a unique “ZIP code” necessary for step C. (Step B) PCR amplification of the target DNA sequences (thermocycling conditions, 95°C for 10 min, 35 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s, and 98°C for 2 min [total, 1.5 h]). Using a common primer pair, target ligated sequence templates labeled with specific “ZIP codes” are multiplied. (Step C) Detection (requiring approximately 1 to 2 h of processing, depending on the number of samples). Amplification products are targeted to specific addresses on the microarray. This targeting is dependent on the specific “ZIP code.” (Step D) Immediate acquisition of images by scanning of the microarray using the array tube reader and immediate interpretation of the acquired pictures by the use of dedicated software.

TABLE 2. Details of discrepancies between conventional PCR plus DNA sequencing results and Check KPC/ESBL genotyping results

Strain	Source or reference	Gene identified by PCR and DNA sequencing	Gene identified by Check KPC/ESBL genotyping	Comment(s)
E. coli DH10B	This study	bla_{SHV-38}	SHV-non-ESBL	Rare chromosomal genotype found in a single *K. pneumoniae* isolate (18); mutation conferring ESBL phenotype not assayed with current microarray primers
K. pneumoniae VA-361[a]	4	bla_{KPC-2}, bla_{TEM-1}, bla_{SHV-11}	KPC, TEM-non-ESBL, SHV-ESBL	Possible production of an SHV-ESBL (e.g., SHV-5/SHV-12)
K. pneumoniae VA-388	4	bla_{KPC-3}, bla_{TEM-1}, bla_{SHV-11}	KPC, TEM-non-ESBL	SHV genes were not detected
K. pneumoniae VA-392[a]	4	bla_{KPC-3}, bla_{TEM-1}, bla_{SHV-11}	KPC, TEM-non-ESBL, SHV-ESBL	Possible production of an SHV-ESBL (e.g., SHV-5/SHV-12)
K. pneumoniae VA-412[a]	4	bla_{KPC-2}, bla_{TEM-1}, bla_{SHV-11}	KPC, TEM-non-ESBL, SHV-ESBL	Possible production of an SHV-ESBL (e.g., SHV-5/SHV-12)
K. pneumoniae VA-414	4	bla_{KPC-3}, bla_{TEM-1}, bla_{SHV-11}	KPC, TEM-non-ESBL, SHV-ESBL	SHV-11 is not an ESBL
K. pneumoniae 111[b]	15	$bla_{TEM-10-like}$, $bla_{SHV-1-like}$	TEM-ESBL, SHV-ESBL	SHV-1 is not an ESBL
K. pneumoniae 438[b]	15	$bla_{TEM-2-like}$, $bla_{SHV-1-like}$	TEM-non-ESBL, SHV-ESBL	SHV-1 is not an ESBL
E. coli 25	This study	$bla_{CTX-M-9-like}$	CTX-M-(IV),[c] TEM-non-ESBL	bla_{TEM} genes were not detected by PCR analysis (including using internal primers); aIEF showed only one β-lactamase band at a pI of 6.7, possibly related to the CTX-M enzyme expression

[a] Analytical isoelectric focusing (aIEF) revealed β-lactamase bands with pIs of 7.6 and 8.2 and initial standard DNA sequencing of bla_{SHV} showed double spikes in amino acid position 238 and/or 240 (4).
[b] Partial DNA sequencing (i.e., from amino acid 35 to 274 for TEMs and from 8 to 249 for SHVs). The bla_{SHV} and bla_{TEM} sequences did not show substitutions conferring an ESBL phenotype.
[c] Number in parentheses following CTX-M designation indicates the family group for CTX-M-type ESBLs (16).

(3–5, 15), whereas the *bla* genes of the remaining isolates were characterized by PCR amplification, standard DNA sequencing, and analytical isoelectric focusing (aIEF) as previously described (4). In this collection, isolates possessed an average of three different *bla* genes (range, one to five; see Table S1 in the supplemental material). The collection also included *K. pneumoniae* ATCC 700603, which produces the SHV-18 ESBL (20), and six *E. coli* DH10B control strains in which single *bla* genes are carried in different plasmid vectors (see Table S1 in the supplemental material).

Genomic DNA of strains was extracted from overnight colonies grown on blood agar (BBL, Sparks, MD) by the use of a DNeasy blood and tissue kit (Qiagen Sciences, Germantown, MD). Microarray assays were performed according to the instructions of the manufacturer (Check-Points Health BV). Briefly, templates of the target *bla* DNA sequences are generated during the ligation step. These templates are then amplified, and the products are hybridized in specific array tubes. Tubes are then inserted in the array tube reader upon completion of the detection reaction, and images are acquired and interpreted with software supplied by the manufacturer (Fig. 1). For 50 isolates, the complete procedure (i.e., from genomic DNA extraction to results) can be performed in approximately 8 h.

Overall, the Check KPC/ESBL system correctly identified representatives of the four *bla* gene families tested, including differentiation between non-ESBL and ESBL genes, in 97 of 106 isolates (91.5%). Specificities of 100% were recorded for the bla_{KPC}, bla_{SHV}, and bla_{CTX-M} genes, whereas one false positive was reported for bla_{TEM} genes (specificity of 96.4%). The system detected all bla_{KPC}-, bla_{TEM}-, and bla_{CTX-M}-possessing isolates, including differentiation of ESBL from non-ESBL bla_{TEM}-containing strains (Table 1). Notably, all bla_{CTX-M} genes detected were classified into the appropriate family group (i.e., group I, CTX-M-1-like; group II, CTX-M-2-like; group III, CTX-M-8-like; group IV, CTX-M-9-like; group V, CTX-M-25/CTX-M-26) according to the classification method of Pitout et al. (16) (see Table S1 in the supplemental material).

Detection and recognition of the bla_{SHV} genes showed 92.5% agreement, with sensitivity and specificity of 98.8% and 100%, respectively (Table 1). Only 1 in 44 bla_{ESBL}-positive strains (i.e., bla_{SHV-38}-positive strains) was not identified (97.7% agreement). SHV-38 is a very rare chromosomal ESBL enzyme (group 2be) that was found in a single clinical isolate. It possesses a unique amino acid substitution (i.e., Ala146Val) and is capable of conferring resistance to ceftazidime and imipenem (18). The amino acid at position 146 is not included in those analyzed by the Check KPC/ESBL system.

Six strains with non-ESBL bla_{SHV} genes were misclassified as ESBLs (Table 2). Notably, three of these were bla_{SHV-11}-positive *K. pneumoniae* isolates (non-ESBL), which showed β-lactamase bands at pIs of 7.6 and 8.2 by aIEF and double spikes at positions 238 and/or 240 in the DNA sequencing traces of the bla_{SHV} gene. This pattern is consistent with the possible production of an SHV-ESBL (along with the non-ESBL SHV-11) that was not detected with a cloning and DNA sequencing method that we previously employed (4). Therefore, bla_{SHV}-positive total agreement and the overall agreement (i.e., all *bla* genes correctly reported) would improve by 2.8% if these three strains were classified as ESBL producers (Table 1).

The data presented above also support the previous observation that standard DNA sequencing of PCR amplification products fails to accurately detect more than one *bla* gene of a given family (4). In particular, many *K. pneumoniae* isolates

possessing both bla_{SHV-11} (non-ESBL) and bla_{SHV-12} (ESBL) genes were initially identified incorrectly as bla_{SHV-11}-positive isolates only with standard DNA sequence analysis (4). In contrast, the microarray can accurately identify the bla_{ESBL} gene (e.g., bla_{SHV-12}) regardless of the coexistence of additional $bla_{non-ESBL}$ genes (e.g., bla_{SHV-1} and/or bla_{SHV-11}) (see Table S1 in the supplemental material).

In conclusion, the results of the present work show that the microarray Check KPC/ESBL system is a highly accurate tool for detection of the clinically important β-lactamase genes found among contemporary Gram-negative organisms. Due to its rapid performance, this platform could be used in epidemiological or infection control studies in which large collections of isolates need to be characterized. Furthermore, the use of Check KPC/ESBL in clinical practice may lead to more appropriate use of antimicrobial agents, reduction of costs, and improved patient outcomes. More-extensive evaluations (e.g., using clinical isolates possessing *bla* genes not tested in this study) are needed to establish the full potential of this methodology for detecting different resistance genes.

This work was supported in part by the Veterans Affairs Merit Review Program (R.A.B.), the National Institutes of Health (grant RO3-AI081036 to R.A.B.), and the Geriatric Research Education and Clinical Center (grant VISN 10 to R.A.B.).

We thank Sarah Drawz for the critical revision of the manuscript and Francesco Luzzaro, Antonio Q. Toniolo, John Quale, David L. Paterson, Gerri S. Hall, and Stephen G. Jenkins for providing clinical isolates. We also thank Check-Points for the technical support and for providing the material necessary for the study.

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