

Evaluation of a DNA Microarray (Check-MDR CT102) for Rapid Detection of TEM, SHV, and CTX-M Extended-Spectrum β -Lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 Carbapenemases[∇]

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The Check-MDR CT102 microarray, aimed at identifying bacteria producing extended-spectrum β -lactamase (ESBL) (SHV, TEM, and CTX-M) and carbapenemase (KPC, OXA-48, VIM, IMP, and NDM-1), was evaluated on a total of 144 Gram-negative strains expressing various β -lactamases. The sensitivity and specificity were 100% for most tested genes, suggesting that this assay allows accurate identification of common ESBL and carbapenemase producers from bacterial cultures.

Extended-spectrum β -lactamases (ESBLs) and carbapenemases are increasingly reported in Gram-negative bacilli (GNB) and represent an emerging public health concern taking into account the paucity of novel antimicrobial drugs (4, 15, 20, 21, 24, 26, 32). The vast majority of ESBLs belong to the TEM-, SHV- and CTX-M-type enzymes (15, 20, 24). 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 known CTX-M enzymes demonstrate expanded-spectrum activity (19, 24). Since ESBL-producing bacteria are often multidrug resistant (MDR), carbapenems represent one of the therapeutic options of last resort for life-threatening infections due to these organisms. Although several mechanisms of carbapenem resistance have been reported, most of the mechanisms are related to the spread of carbapenemases belonging to Ambler class A (KPCs), class B (VIMs, IMPs, and NDM-1), and class D (OXA-48) β -lactamases (4, 10, 14, 17, 21, 26). These enzymes compromise the clinical efficacy of almost the whole armamentarium of antimicrobial drugs, leaving clinicians with only a limited number of “last-line” agents such as colistin (4, 21, 26, 32, 36).

Laboratory detection of ESBL-producing bacteria remains a challenge for the microbiology laboratory and is important both to avoid clinical failure due to inappropriate antimicrobial therapy and to prevent nosocomial outbreaks. However, routine phenotypic testing may fail to identify ESBL producers, and it also usually delays the final report by an additional 24 h (1). Several molecular assays such as real-time PCR targeting ESBL genes have been proposed for the detection of CTX-M-type ESBLs (2, 19, 23, 25, 34), but these techniques

are not suited for the rapid detection of TEM and SHV ESBLs in (daily) routine use, since sequencing is required to distinguishing ESBLs from the non-ESBL variants (18, 27).

The detection of carbapenemases primarily on the basis of phenotypic testing may sometimes be difficult, since the MICs of carbapenem drugs may remain in the susceptibility range (5, 22). Detection and identification of these carbapenem-resistant isolates may be improved significantly by the use of automated susceptibility testing systems (35). Yet, specific gene identification using molecular techniques remains the gold standard for their identification (22), and several PCR approaches have been proposed (3, 8, 13, 16, 22). Identification of asymptomatic stool carriage of carbapenemase producers is mostly based on usage of screening selective culture media such as ChromID ESBL (bioMérieux, Marcy-l’Etoile, France) or CHROMagar KPC (CHROMagar, Paris, France) (5, 22, 31). However, even though these media may be easy to use, it usually requires an additional 48 h before the carrier status of a patient can be definitively established (22, 28). In order to control the spread of carbapenemase-producing bacteria in hospitalized patients, effective infection control measures (i.e., rapid isolation and/or cohorting of colonized or infected patients) and controlled antibiotic usage must be associated with the use of rapid and sensitive molecular biology-based diagnostic assays, as suggested for the control of KPC-producing isolates (13).

Microarray technology has recently been developed for the typing of *Salmonella enterica* isolates (33). This technology has the potential to detect an almost unlimited number of genes within a single reaction. A commercial microarray technology-based DNA test, the Check-KPC ESBL microarray (Check-Points Health BV, Wageningen, the Netherlands), aimed at identifying TEM-, SHV-, and CTX-M-type ESBLs as well as KPC-type carbapenemases, has recently been evaluated, and it was found to yield an excellent sensitivity and specificity (7, 11, 18). Here, we have evaluated a further refinement of this

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microarray, the Check-MDR CT102 microarray (formerly known as the Check-Carba ESBL microarray), that in addition to the previously detected genes, includes other clinically relevant carbapenemase-coding genes, such as OXA-48, VIM, and IMP, as well as the rapidly emerging NDM-1.

A total of 107 members of the family *Enterobacteriaceae*, 28 *Pseudomonas* isolates, and 9 *Acinetobacter* isolates possessing different β -lactamase genes were tested (Tables 1 and 2). The majority of the strains had previously been well characterized with respect to their β -lactamase genes (9, 18, 22, 29) (Tables 1 and 2), whereas the remaining β -lactamase genes for some isolates were characterized by PCR amplification, standard DNA sequencing, and analytical isoelectric focusing (IEF) as previously described (18, 30). In this collection, isolates possessed an average of three different β -lactamase genes (range, one to five isolates). This collection also included reference bacterial isolates displaying a wild-type resistance phenotype, expressing no β -lactamase gene or only the naturally encountered β -lactamase genes, and several isolates producing other resistance genes that were used as negative-control strains (18). Furthermore, this collection of isolates expressed various resistance levels for a given carbapenemase gene, as deduced by MIC values (6).

Whole-cell DNAs were extracted from overnight bacterial cultures using QiaAmp DNA minikit (Qiagen, Les Ulis, France). Microarray assays were performed according to the instructions of the manufacturer and as previously outlined (7, 18). Briefly, templates of the target *bla* DNA sequences were generated during a multiplex ligation detection reaction step. These templates are then amplified, and the products are hybridized in specific array tubes. The tubes are then inserted in the single-channel ATR03 array tube reader upon completion of the detection reaction, and images are acquired and interpreted with the software supplied by the manufacturer (Check-Points). This software automatically translates the Check-MDR CT102 microarray data into the presence or absence of a specific β -lactamase gene. Typical DNA microarray pictures obtained with the Check-MDR CT102 microarray setup are shown in Fig. 1. The analysis time from genomic DNA extraction to results (7 to 8 h) is relatively short compared to the barely predictable time scale of classical PCR followed by sequencing.

The system was evaluated on well-characterized clinical isolates from different geographic origins and expressing several β -lactamase genes. The results are summarized in Tables 1 and 2. Overall, the Check-MDR CT102 microarray system correctly identified representatives of the three ESBL gene families tested, including differentiation between non-ESBL and ESBL genes, in all isolates (100%). A specificity of 100% was recorded for the detection of ESBL genes of the *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} types. The assay was able to detect all *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}-type ESBLs, even in strains simultaneously harboring non-ESBL *bla*_{TEM}- or *bla*_{SHV} genes (Table 1). Notably, all *bla*_{CTX-M} genes that were detected were classified into the appropriate family group (i.e., CTX-M-1-like, CTX-M-2-like, CTX-M-9-like, and CTX-M-8/25/26-like) (15, 24). The different probes did not cross-react with the chromosomally encoded K1 β -lactamases of *Klebsiella oxytoca*, which is genetically related to CTX-M-type β -lactamases (24). Furthermore, none of the other β -lactamase genes cross-re-

acted with the probes used in the assay, suggesting an excellent specificity.

The naturally encoded SHV- β -lactamase was accurately detected in all *Klebsiella pneumoniae* isolates, given that SHV was present. However, as previously shown (12, 18), *bla*_{OKP} and *bla*_{LEN} genes which may be encountered in 10% of *K. pneumoniae* isolates were not detected by the microarray. However, since neither plasmid-encoded nor ESBL variants of LEN or OKP have been reported as yet, we would consider that these enzymes are not clinically relevant and that their detection failure is not a clinical issue.

The data presented above also support the previous observations that standard DNA sequencing of PCR amplification products fails to accurately detect the simultaneous presence of more than one *bla* gene of a given family (4). In particular, several *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 when standard DNA sequence analysis was used (4). In contrast, the microarray allowed the accurate identification of specific ESBL genes (e.g., *bla*_{SHV-12}) regardless of the coexistence of additional non-ESBL genes (e.g., *bla*_{SHV-1} and/or *bla*_{SHV-11}) (Table 1). One *bla*_{TEM-1} gene was reproducibly not identified in one *Acinetobacter baumannii* isolate, likely the result of poor DNA extraction or of plasmid instability. Real-time SYBR green PCR assay yielded a weak (late) positive amplification signal for the *bla*_{TEM-1} gene, suggesting that there was a small amount of template DNA (data not shown). An intrinsic limitation of this type of genotypic assay is the limited number of ESBLs (or ESBL families) that may be detected. The array was designed to include the most prevalent ESBLs, resulting in coverage of 95% (84/88) of the TEM ESBLs and 77% (27/35) of the SHV ESBLs described in the Lahey database and 100% of CTX-M-type ESBLs (7), but it misses minor ESBLs, such as VEB, PER, and GES (20). Another limitation of this approach is the identification at the enzyme “family” level only.

The Check-MDR CT102 microarray detected all the carbapenemase genes in all the bacterial isolates tested with the exception of one *K. pneumoniae* isolate and one *Enterobacter cloacae* isolate (Table 2), where failure to detect *bla*_{KPC} was likely the result of plasmid instability, as previously shown (18). In these isolates, *bla*_{KPC} was present on a small 12-kb plasmid lacking partitioning functions (10). Specificities and sensitivities of 100% were recorded for *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}, and *bla*_{OXA-48} genes, whereas for the *bla*_{KPC} gene, they were 100% and 85%, respectively. The positive and negative predictive values were both 100% for the *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}, and *bla*_{OXA-48} carbapenemase genes, and the positive and negative predictive values for *bla*_{KPC} genes were 100% and 98%, respectively.

Overall, this assay correctly identified 70 ESBL genes among the three targeted classes in 66 ESBL-producing isolates and was capable of distinguishing between ESBL and non-ESBL variants of TEM and SHV, even when both variants were present in the same isolate. Furthermore, it was capable of correctly identifying representatives of the five targeted carbapenemase genes in 83/85 carbapenemase producers. All but two isolates (2 KPC producers) could be accurately identified, irrespective of their MICs for carbapenems. Some isolates produced up to five different genes that were targeted by the

TABLE 1. DNA array results on various clinical isolates harboring ESBL genes

Species ^a	No. of isolates	No. of isolates ^b with the <i>bla</i> _{CTX-M} gene by:		% agreement for <i>bla</i> _{CTX-M} results ^d		No. of isolates ^c with <i>bla</i> _{SHV} gene by:		% agreement for non-ESBL <i>bla</i> _{SHV} results		No. of isolates ^e with <i>bla</i> _{TEM} gene by:		% agreement for non-ESBL <i>bla</i> _{TEM} results		No. of isolates ^f with non-ESBL <i>bla</i> _{TEM} gene by:		% agreement for non-ESBL <i>bla</i> _{TEM} results		Total no. of strains that agree/total no. of strains ^g		% agreement for all strains	
		PCR ^h		Array ⁱ		PCR		Array		PCR		Array		PCR		Array		PCR		Array	
		39	51	39	51	6	15	6	100 ^j	53	51	96	0	0	41	41	100	55/57	96	100	55/57
Enterobacteria																					
<i>Klebsiella pneumoniae</i>	57	39	51	39	51	6	15	6	100	53	51	96	0	0	41	41	100	55/57	96	100	55/57
<i>Escherichia coli</i>	25	10	10	10	10	0	0	0	100	0	0	100	2	2	9	9	100	25/25	100	100	25/25
<i>Enterobacter cloacae</i>	7	1	1	1	1	2	2	2	100	0	0	100	0	0	0	0	100	7/7	100	100	7/7
<i>Serratia marcescens</i>	1	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	100	1/1	100	100	1/1
<i>Citrobacter freundii</i>	4	1	1	1	1	0	0	0	100	0	0	100	1	1	2	2	100	4/4	100	100	4/4
<i>Enterobacter aerogenes</i>	3	0	0	0	0	0	0	0	100	0	0	100	1	1	0	0	100	3/3	100	100	3/3
<i>Providencia stuartii</i>	2	0	0	0	0	0	0	0	100	0	0	100	0	0	1	1	100	2/2	100	100	2/2
<i>Proteus mirabilis</i>	3	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	100	3/3	100	100	3/3
<i>Klebsiella oxytoca</i>	5	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	100	5/5	100	100	5/5
Nonfermenters																					
<i>Pseudomonas aeruginosa</i>	25	0	0	0	0	7	7	7	100	0	0	100	0	0	7	7	100	25/25	100	100	25/25
<i>Pseudomonas putida</i>	3	0	0	0	0	0	0	0	100	0	0	100	0	0	1	1	100	3/3	100	100	3/3
<i>Acetobacter baumannii</i>	8	0	0	0	0	0	0	0	100	0	0	100	0	0	2	2	50	7/8	89	50	7/8
<i>Acetobacter junii</i>	1	0	0	0	0	0	0	0	100	0	0	100	0	0	1	1	100	1/1	100	100	1/1
Total	144	51	51	39	51	15	15	100 ^k	100 ^l	53	51	96	4	4	64	64	100 ^m	143/144	99	100	143/144

^a The isolates tested include control isolates producing VEB-1 (1 isolate), PER-1 (1 isolate), GES-7 (1 isolate), ACC-1 (1 isolate), ACT-1 (1 isolate), CMY-2 (1 isolate), OXA-1 (5 isolates), OXA-18 (1 isolate), OXA-23 (1 isolate), and KOCY (5 isolates).

^b Includes isolates producing CTX-M-1 (1 isolate), CTX-M-2 (2 isolates), CTX-M-9 (7 isolates), CTX-M-14 (1 isolate), CTX-M-15 (37 isolates), and CTX-M-19 (1 isolate).

^c Includes isolates producing SHV-2 (2 isolates), SHV-2a (3 isolates), SHV-5 (7 isolates), SHV-12 (2 isolates), and SHV-30 (1 isolate).

^d Includes isolates producing SHV-1 (22 isolates), SHV-11 (15 isolates), SHV-28 (15 isolates), OKP-A/B (1 isolate), and LEN-1 (1 isolate).

^e Includes isolates producing TEM-3 (1 isolate), TEM-11 (1 isolate), TEM-24 (1 isolate), and TEM-52 (1 isolate).

^f Includes isolates producing TEM-1 (63 isolates) and TEM-2 (1 isolate).

^g Total number of strains correctly identified taking all results into account.

^h Results obtained with classical PCR/sequencing.

ⁱ Results obtained with the Check-MDR CT102 microarray.

^j Percent agreement between the two methods.

^k A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for CTX-M detection.

^l A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for SHV ESBL detection.

^m A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for TEM ESBL detection.

TABLE 2. DNA array results on various clinical isolates harboring carbapenemase genes

Species ^a	No. of isolates	No. of isolates ^b with the <i>bla</i> _{KPC} gene by:		% agreement for <i>bla</i> _{KPC} results ^c	No. of isolates ^d with the <i>bla</i> _{OXA-48} gene by:		% agreement for <i>bla</i> _{OXA-48} results	No. of isolates ^e with the <i>bla</i> _{NDM-1} gene by:		% agreement for <i>bla</i> _{NDM-1} results	No. of isolates ^f with the <i>bla</i> _{IMP} gene by:		% agreement for <i>bla</i> _{IMP} results	No. of isolates ^g with the <i>bla</i> _{VIM} gene by:		% agreement for <i>bla</i> _{VIM} results	Total no. of strains that agree/total no. of strains ^h	% agreement for all strains
		PCR ^h	Array ^j		PCR	Array		PCR	Array		PCR	Array		PCR	Array			
Enterobacteria																		
<i>K. pneumoniae</i>	57	8	7	88	17	17	100	18	18	100	2	2	100	1	1	100	56/57	98
<i>E. coli</i>	25	3	3	100	0	0	100	2	2	100	4	4	100	1	1	100	25/25	100
<i>E. cloacae</i>	7	2	1	50	1	1	100	0	0	100	0	0	100	0	0	100	6/7	86
<i>S. marcescens</i>	1	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	1/1	100
<i>C. freundii</i>	4	0	0	100	1	1	100	1	1	100	0	0	100	0	0	100	4/4	100
<i>E. aerogenes</i>	3	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	3/3	100
<i>P. stuartii</i>	2	0	0	100	1	1	100	0	0	100	0	0	100	0	0	100	2/2	100
<i>P. mirabilis</i>	3	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	3/3	100
<i>K. oxytoca</i>	5	0	0	100	0	0	100	0	0	100	0	0	100	2	2	100	5/5	100
Nonfermenters																		
<i>P. aeruginosa</i>	25	0	0	100	0	0	100	0	0	100	3	3	100	12	12	100	25/25	100
<i>P. putida</i>	3	0	0	100	0	0	100	0	0	100	1	1	100	2	2	100	3/3	100
<i>A. baumannii</i>	8	0	0	100	0	0	100	0	0	100	2	2	100	0	0	100	8/8	100
<i>A. junii</i>	1	0	0	100	0	0	100	0	0	100	1	1	100	0	0	100	1/1	100
Total	144	13	11	85 ^k	20	20	100 ^l	21	21	100 ^m	13	13	100 ⁿ	18	18	100 ^o	142/144	99

^a The isolates tested include control isolates producing VEB-1 (1 isolate), PER-1 (1 isolate), GES-7 (1 isolate), ACC-1 (1 isolate), ACT-1 (1 isolate), CMY-2 (16 isolates), DHA-2 (1 isolate), OXA-1 (5 isolates), OXA-18 (1 isolate), OXA-23 (1 isolate), and KOXY (5 isolates).

^b Includes isolates producing KPC-2 (12 isolates) and KPC-3 (1 isolate).

^c Includes OXA-48-producing bacterial isolates with variable MICs (0.5 µg/ml to >64 µg/ml).

^d Includes NDM-1-producing bacterial isolates with variable MICs (2 µg/ml to >64 µg/ml).

^e Includes isolates producing IMP-1 (8 isolates), IMP-4 (1 isolate), IMP-8 (2 isolates), and IMP-13 (2 isolates).

^f Includes isolates producing VIM-1 (3 isolates), VIM-2 (9 isolates), VIM-3 (2 isolates), VIM-4 (2 isolates), and VIM-19 (2 isolates).

^g Total number of strains correctly identified taking all results into account.

^h Results obtained with classical PCR/sequencing.

ⁱ Results obtained with the Check-MDR CT102 microarray.

^j Percent agreement between the two methods.

^k A sensitivity of 85%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 98% for KPC detection.

^l A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for OXA-48 detection.

^m A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for NDM-1 detection.

ⁿ A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for IMP detection.

^o A sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100% for VIM detection.

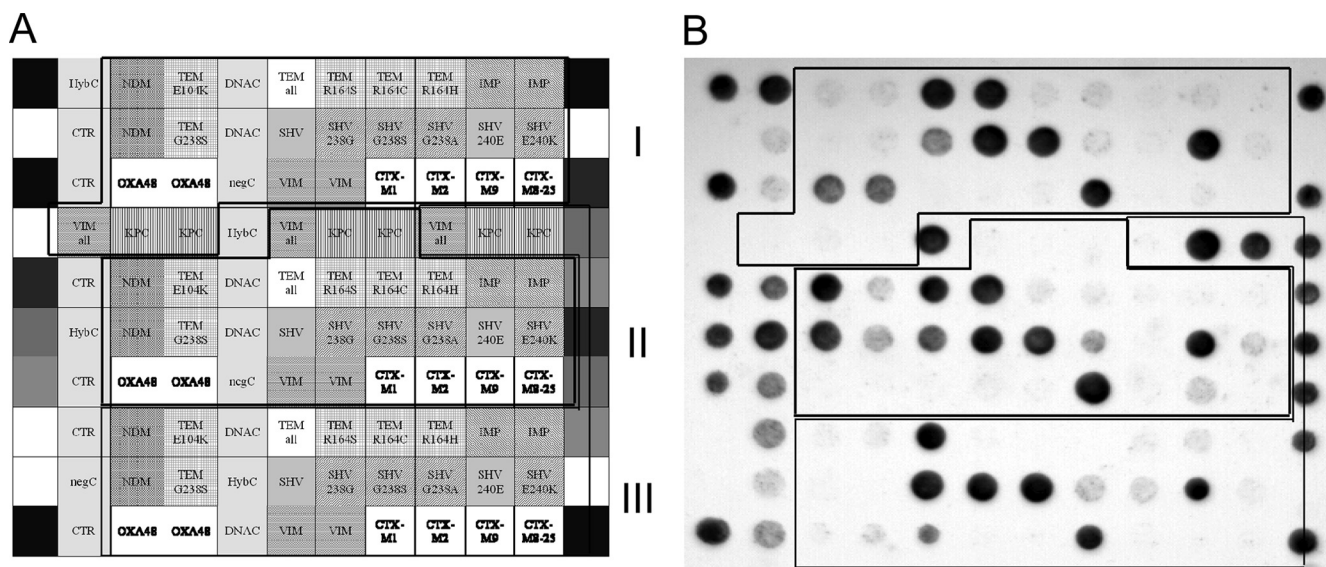


FIG. 1. Typical DNA microarray pictures obtained with the Check-MDR CT102 microarray setup. This format uses a DNA microarray fixed at the bottom of a microreaction vial. The microarray consists of unique complementary (cZIP) oligonucleotides targeting individual probes. When hybridization of the PCR-amplified ligation products to the microarray is complete, colorimetric detection of the positive reactions is initiated. Panels in the array are outlined. Each panel defines the typing results of one strain and consists of control spots and specific marker spots, which are numbered from 1 to 96. (A) Theoretical display of the array probes for strain 1 (panel I), strain 2 (panel II), and strain 3 (panel III). (B) Array results for *K. pneumoniae* HPA-1 ([9]; OXA-48, SHV-1, TEM-1, CTX-M-15; panel I), *K. pneumoniae* Afr 2 ([22]; NDM-1, SHV-1, TEM-1, CTX-M-15, CMY-2; panel II), and *K. pneumoniae* 16 ([10]; KPC-2, SHV-1 CTX-M-15; panel III). HybC, hybridization control; CTR, control; negC, negative control; DNAC, DNA control.

array and were correctly identified. The Check-MDR CT102 microarray was found to be robust, specific, and sensitive. It gave satisfactory results on most DNA extracts of enterobacterial, *Pseudomonas* spp., and nonfermenter isolates. Nevertheless, the present format of the Check-MDR CT102 microarray is more suited for members of the family *Enterobacteriaceae*, since several important genes are missing for an integrated approach to the detection of major transferable β -lactamases found in non-fermenters, such as OXA-23, OXA-24, and OXA-58 clusters of carbapenemases for *A. baumannii* or the several OXA, PER, GES, VEB, and BEL-1 ESBLs for *Pseudomonas aeruginosa* and *A. baumannii* (20).

The ability to rapidly distinguish narrow-spectrum β -lactamases (e.g., SHV-11 and TEM-1) from 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 display resistance to extended-spectrum oxymino-cephalosporins and to aztreonam (24). Since coresistance to quinolones and to aminoglycosides is frequently observed among ESBL producers, carbapenems represent the major and last therapeutic options for life-threatening infections due to these organisms (24). Finally, with the increased use of carbapenems, increasing ESBL or AmpC producers resistant to carbapenems by porin deficiency may be selected. The Check-MDR CT102 microarray is capable of differentiating them from true carbapenemase producers.

Use of the Check-MDR CT102 microarray on a daily routine basis is still hampered by the low prevalence of carbapenem-producing isolates and the high price-to-analysis ratio.

Furthermore, even though most of the required equipment to perform this assay is present in standard molecular laboratories, an array reader would have to be purchased, which may be another limiting factor. However, due to its rapid performance, this platform could be used in epidemiological or infection control studies as a reliable molecular confirmatory test to identify ESBL or carbapenemase genes in bacteria displaying frank resistance or only reduced susceptibilities to expanded-spectrum cephalosporins or to carbapenems. Indeed, carbapenemase-producing enterobacterial isolates may remain susceptible to carbapenems (14, 17, 21).

In conclusion, the Check-MDR CT102 microarray is a highly accurate tool for detection of clinically important β -lactamase genes found among currently spreading MDR members of the *Enterobacteriaceae*. Its use in clinical practice may contribute to better knowledge of the local epidemiology of β -lactam resistance genes and to more appropriate use of antimicrobial agents, reduction of costs, and improved patient outcome.

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