Evaluation of a commercial microarray as a confirmation test for the presence of extended-spectrum β-lactamases in isolates from the routine clinical setting

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Abstract

Since the diagnostic characteristics of the Check-KPC ESBL microarray as a confirmation test on isolates obtained in a routine clinical setting have not been determined, we evaluated the microarray in a random selection of 346 clinical isolates with a positive ESBL screen test (MIC >1 mg/L for cefotaxime or ceftazidime or an ESBL alarm from the Phoenix or Vitek-2 expert system) collected from 31 clinical microbiology laboratories in the Netherlands in 2009. Using sequencing as the reference method the sensitivity of the microarray was 97% (237/245), the specificity 98% (97/99), the positive predictive value 99% (237/239) and the negative predictive value 92% (97/105).

Keywords: Check-points, clinical setting, confirmation test, ESBL, microarray

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Worldwide, the prevalence of extended-spectrum β-lactamases (ESBLs) is increasing at an alarming rate [1]. For infection control precautions and the choice of adequate antibiotic therapy, accurate and rapid detection of ESBLs is important.

In Enterobacteriaceae, the most prevalent ESBL gene families are CTX-M, TEM and SHV [1]. For rapid detection of those ESBL families, a microarray system has been developed (Check-KPC ESBL, Check-Points B.V., Wageningen, the Netherlands) [2]. This system uses ligation-mediated amplification, combined with detection of amplified products on a microarray to detect the various CTX-M groups (CTX-M group 1, 2, 9, or combined 8/25) and the ESBL-associated single-nucleotide polymorphisms (SNPs) in TEM and SHV variants. The assay can not provide a Lahey type number for TEM and SHV genes (e.g. TEM-6 or SHV-2), but reports to which group they belong. Compared with phenotypic detection methods, this array system is faster (obtaining results within one working day) and provides information on the (combination of) TEM, SHV or CTX-M groups present, which may be used for epidemiological or infection control purposes.

Evaluation of this microarray has been performed on collections of isolates expressing a wide variety of β-lactamase genes [2–4]. High sensitivities (95–100%) and specificities (96–100%) were found in these studies. The aim of this study was to determine the accuracy of the Check-KPC ESBL microarray as a confirmatory test of ESBLs in the routine laboratory setting (i.e. on randomly selected clinical isolates with a positive ESBL screen test). Therefore 346 clinical isolates collected in a national ESBL surveillance study in the Netherlands were included. In this survey, 31 clinical microbiology laboratories collected all *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Enterobacter* spp. isolates with a positive ESBL screen test according to the national guidelines (MIC >1 mg/L for cefotaxime or ceftazidime or an ESBL alarm from the Phoenix or Vitek-2 expert system) from 1 February until 1 May 2009 (http://www.nvmm.nl/richtlijnen/esbl-screening-en-confirmatie). Of the 1418 collected isolates, the first 25 non-repeat isolates (one per patient) per participating laboratory were selected for genotypic analysis, resulting in a collection of 692 isolates. The accuracy of the microarray was evaluated on a computer-generated random sample of 50% of those isolates (n = 346). There were no significant differences between the species distribution in the random sample and the total collection. As a reference test, we used the presence of ESBL genes determined by PCR and DNA sequencing on the same DNA batch as used for the microarray [2]. In case of presence of multiple TEM- and SHV-alleles, base calling for both alleles at positions in the sequence chromatogram that

showed double peaks in the forward and reversed strand was resolved manually.

Microarray analysis was performed according to the instructions of the manufacturer, and interpreted using software version 20090508T164015R74 (Check-Points). DNA isolation was performed using the Ultraclean Microbial DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the instructions of the manufacturer. In the case of a positive ESBL phenotype according to the participating laboratory (confirmation was performed according to the national guidelines using ESBL Etest (BioMérieux, Marcy l'Etoile, France) or combidisks with ceftazidime, cefotaxime and/or cefepime with and without clavulanic acid) and an ESBL-negative result of PCR and DNA sequencing for CTX-M, TEM and SHV ESBL genes, additional PCRs were performed to detect the presence of rare ESBL families such as PER, GES and VEB β-lactamase genes [5].

Statistical analyses were performed using SPSS 15.0 (IBM Inc., Armonk, NY, USA) and Microsoft Excel 2003 (Microsoft, Redmond, WA, USA).

Two of the 346 included isolates were excluded from analysis because DNA sequence results could not be obtained. Of the remaining 344 isolates, 75% were *E. coli* (n = 257), 10% *K. pneumoniae* (n = 35), 10% *Enterobacter cloacae* (n = 33), 3% *P. mirabilis* (n = 10) and 3% *K. oxytoca* (n = 9). Based on PCR and sequencing, 245 isolates were ESBL positive and 99 ESBL negative. Among the 245 ESBL-positive isolates, in total 255 ESBL genes were identified: 209 CTX-M, 28 SHV, 16 TEM, 1 GES and 1 PER.

The sensitivity of the microarray for the detection of an ESBL was 97% (237/245), the specificity 98% (97/99), the positive predictive value 99% (237/239) and the negative predictive value 92% (97/105).

For 95% (228/239) of the isolates with an ESBL positive microarray result the outcome of the microarray was in accordance with the sequencing results. In Table 1 the discrepancies in the 11 isolates are specified.

A false-negative result was obtained in eight isolates. In six isolates, a CTX-M-1 group ESBL gene was not detected (four CTX-M-15/28 positive isolates, one CTX-M-1 positive isolate, and one CTX-M-22 positive isolate), even after repeating the test. These six represented 3% (6/182) of all CTX-M-1 group positive isolates in the collection (three *E. coli*, two *K. pneumoniae* and one *E. cloacae*). This finding is in contrast to previous studies, where only failures in the detection of TEM and SHV genes were reported, and worrisome because CTX-M-1 group enzymes, especially CTX-M-15/28, are the most prevalent ESBLs worldwide [6]. The reason is unknown, but may be explained by chance, because 74% (182/245) of the isolates in this collection harboured a CTX-M-1 group gene, a limited sensitivity of the CTX-M-1 group-specific probe or a modification of the interpretation software resulting in an alteration of the detection limit. The other two false negative isolates contained an ESBL gene not included in the design of the array (one PER and one GES producing isolate).

A false-positive result was obtained in two isolates containing a TEM-1 gene. However, in these isolates a TEM-17 and a TEM-19 group ESBL gene were identified by the array next to a non-ESBL TEM, and both had an ESBL-positive phenotype as determined by ESBL Etest. Therefore, these false-positive results may be explained by the limitation of using

TABLE 1. Comparison of DNA sequence and microarray results

ESBL-genotype based on DNA sequencing (n)	Isolates with concordant results n (%)	Isolates with discrepant results		
		n (%)	Outcome sequencing[a]	Outcome microarray
Negative (99)	97 (98)	2 (2)	1 TEM-1 (non-ESBL)	1 TEM-17 group (ESBL)
			1 TEM-1 (non-ESBL)	1 TEM-19 group (ESBL)
CTX-M-family (199)	190 (95)	9 (5)	4 CTX-M 15/28 (CTX-M-1 group)	4 Negative
			1 CTX-M-1 (CTX-M-1 group)	1 Negative
			1 CTX-M-22 (CTX-M-1 group)	1 Negative
			1 CTX-M-65 (CTX-M-9 group)	1 CTX-M-1 group
			1 CTX-M-15/28 (CTX-M-1 group)	1 CTX-M-1 group, CTX-M-8/25 group
			1 CTX-M-15/28, TEM-1 (CTX-M-1 group, TEM non-ESBL)[b]	1 CTX-M-1 group, TEM-19 group
SHV-family (20)	18 (90)	2 (10)	1 SHV-12 (SHV-4 group)	1 SHV-2 group
			1 SHV-12 (SHV-4 group)	1 SHV-4 group, CTX-M-9 group
TEM-family (14)	13 (93)	1 (7)	1 TEM-19 (TEM-19 group)	1 TEM-3 group
Combination of genes (10)	7 (70)	3 (30)	1 CTX-M-1, SHV-12 (CTX-M-1 group, SHV-4 group)	1 CTX-M-1 group
			1 SHV-12, TEM-25 (SHV-4 group, TEM-19 group)	1 SHV-31 group, TEM-19 group
			1 CTX-M-15/28, SHV-12 (CTX-M-1 group, SHV-4 group)	1 CTX-M-1 group, SHV-2 group
Other ESBL genes (2)	0 (0)	2 (100)	1 PER-5	1 Negative
			1 GES-1	1 Negative

[a]Between brackets the array group to which the gene belongs is noted.
[b]Beside the noted CTX-M gene, a TEM ESBL could not be confirmed and only a TEM non-ESBL was found by sequencing.