

#C2-693

# DNA Micro-Array Test combined with PCR-sequencing: A useful Tool for Rapid Identification of ESBL Genes

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## Revised Abstract

**Background:** Extended Spectrum  $\beta$ -lactamases (ESBLs) constitute the major cause of resistance to  $\beta$ -lactams among *Enterobacteriaceae*. TEM and SHV-type ESBLs have been dominant but during the past decade, rapid and extensive spread of CTX-M enzymes has been described worldwide. Due to the high diversity of these enzymes, a rapid and accurate method is crucial for epidemiological surveys. **Methods:** We tested 1,130 isolates collected from the SMART program (2008-2009) including a majority of *E. coli* (64.8%) and *K. pneumoniae* (30.1%) and *E. cloacae*, *E. aerogenes*, *K. oxytoca* and *P. mirabilis* (5.1%). A new commercial molecular test "Check-Points (CP) Check-KPC ESBL" combined with PCR-sequencing, allowing a fast and complete characterization of SHV, TEM and CTX-M-type ESBLs, was used. **Results:** Isolates were collected from patients with intra-abdominal infections from Asia (37.7%), Europe (27.3%), Latin America (20.9%), North America (6.3%), South Pacific (4.9%), Middle East (2%) and Africa (0.9%).

ESBL/Region	Africa	Asia	Europe	Latin America	Middle East	North America	South Pacific	Total
SHV	1.5	18	37.5	16	1.5	19	6.5	100
SHV-12	1.6	17.5	34.1	13.5	1.6	23	8.7	100
Other	-	11.8	58.8	14.7	2.9	11.8	-	100
TEM	-	17.6	29.4	41.2	-	5.9	5.9	100
TEM-52	-	25	12.5	62.5	-	-	-	100
Other	-	11.1	44.4	22.2	-	11.1	11.1	100
CTX-M	0.8	42.5	24.6	22.1	2	3.2	4.8	100
CTX-M15	0.8	42.2	25.3	21.3	2.1	3.3	4.9	100
Other	1	43.2	23.2	23.5	1.9	2.9	4.4	100

CTX-M-type ESBLs are currently the most common enzymes reported worldwide, especially in Asia (42.5%), Europe (24.6%) and Latin America (22.1%). SHV-type ESBLs were more common in Europe (37.5%) than in other regions. The most common enzymes were CTX-M15 (Asia), SHV-12 (Europe) and TEM-52 (Latin America). **Conclusions:** In an environment in which ESBLs are becoming an increasing threat, tools for the rapid identification of these rapidly evolving  $\beta$ -lactamases are needed both to aid in infection control and for epidemiological studies.

## Introduction

Extended-spectrum  $\beta$ -lactamases (ESBLs) are a worldwide public health problem: (7) they have rapidly emerged worldwide in *Enterobacteriaceae* they are considered to be one of the most important antibiotic resistance mechanisms. The majority of ESBLs belong to TEM-, SHV- and CTX-M types: (<http://www.lahey.org/studies/>)

- More than 180 TEM-type and 130 SHV-type  $\beta$ -lactamases have been identified worldwide
- The main mutations conferring the ESBL phenotype are found at the following positions: TEM: 104, 164, 238 and 240 SHV: 238 and 240
- All the CTX-M enzymes are ESBLs: more than 90 CTX-M variants, divided into 5 five groups (CTX-M-1, 2, 9 and B/25), have been identified (2).

Resistance to carbapenems due to the production of KPC enzymes in *Enterobacteriaceae* is a growing issue as well (3). To date, 11 KPC-variants have been described.

- Optimal detection of ESBLs/KPC is now highly important: (4)
- ESBLs detection is primarily based on phenotypic testing and standard molecular tests tend to be used to characterize isolates with these genes. PCR-sequencing is the most widely method used.
- Detection of KPC enzymes is difficult using phenotypic methods

The huge diversity of these enzymes makes their detection truly crucial to routinely monitor their prevalence and worldwide distribution. Fast and reliable molecular techniques which could be used in clinical microbiology laboratories are essential.

## Materials & Methods

### Bacterial strains:

The Study for Monitoring Antimicrobial Resistance Trends (SMART) is a global longitudinal antimicrobial surveillance study that has been monitoring susceptibility of Gram-negative aerobic pathogens from intra-abdominal infections (IA) since 2002.

In this study, 1,130 recent isolates (2008-2009) phenotypically identified as ESBL-positive from the SMART program were tested: *E. coli* (64.8%), *K. pneumoniae* (30.1%), *K. oxytoca* (1.1%), *E. cloacae* (2.6%), *E. aerogenes* (0.4%), and *P. mirabilis* (1%).

ESBL testing was done according to CLSI guidelines (1), looking for a  $\geq 5$ -doubling dilution decrease in MIC of ceftazidime or ceftotaxime in the presence of clavulanic acid.

7 control strains, representing the ESBL targeted (SHV, TEM, CTX-M1, 2, 9, B/25) by the Check-Points method and PCR, were used in this study.

## Material & Methods(cont.)

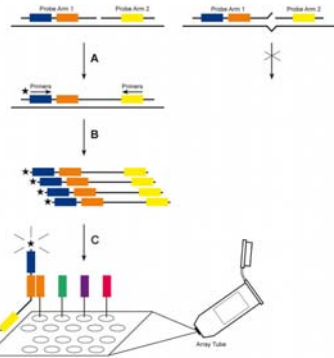
### DNA sample preparation:

Whole DNAs were extracted with the QIAAGEN QiaAmp DNA mini kit using the QIAcube instrument (12 samples an hour)



### DNA micro-array method:

Figure 1: Principle of the CP method:



### A-Ligation Detection Reaction

Probe contains:

Probe Arm 1:

A specific sequence at the 5' with universal primers (blue box) linked to a unique ZIP code (orange box)

Probe Arm 2:

A specific sequence at the 3' with universal primers (yellow box)

Probes are hybridized to target DNA and only in case of a perfect match, the probes are joined by a ligase. Critical mismatches in the target sequence will cause ligation to fail, leaving the probes apart.

### B-Polymerase Chain reaction (PCR)

Successful ligation products are amplified by PCR using a single pair of universal primers annealing to complementary sequences included in the probes (blue and yellow boxes)

### C-Hybridization and Detection

Unique ZIP codes (orange box) assigned to each probe will be specifically captured by complementary oligonucleotides spotted on the microarray and will be detected using a biotin label incorporated in one of the PCR primers.

The final results are obtained using a specific reader (ATRO3) and software:



The system can be multiplexed with many different probes, each bearing a different ZIP code.

### PCR-sequencing:

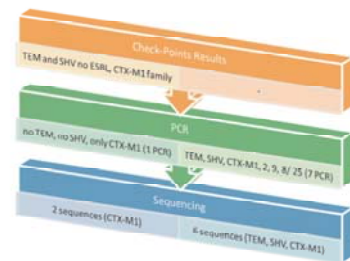
Primers used for detection and sequencing of the blaCTX-M, blaSHV, blaTEM and blaKPC were described previously (5, 6, 8, 9)

Both strands of the PCR products were sequenced using an Applied Biosystems sequencer (ABI 3730)

Sequences were analyzed using the SeqScape software

## Advantages of Check-ESBL KPC combined with PCR-sequencing

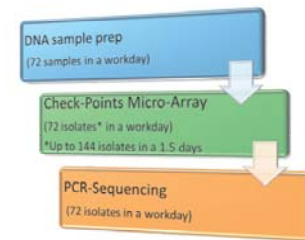
Figure 1: Example for an isolate carrying only the CTX-M1 enzyme:



The Check-Points array results allowed:

- avoiding sequencing of the non-ESBL enzymes
- orienting sequencing for the CTX-M groups.

Figure 2: Time to response for complete characterization of 72 isolates



3 working days for complete characterization of 72 isolates

## Results

### Worldwide distribution of ESBL genes

Figure 3: Distribution of ESBL genes among 1,119 ESBL-producing isolates

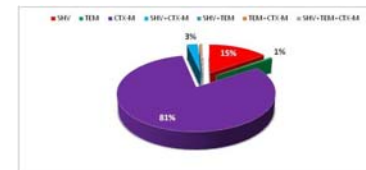
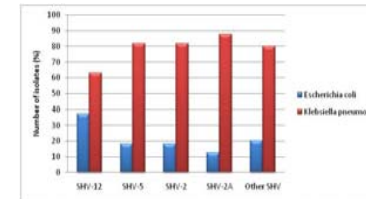


Figure 4: Distribution of the most common SHV genes among E. coli and K. pneumoniae isolates



-SHV-12 (63%)  
-SHV-5 (17%)  
-SHV-2 (5%) and SHV-2A (4%)

Figure 5: Worldwide distribution of SHV-12 genes among 1,119 ESBL-producing isolates

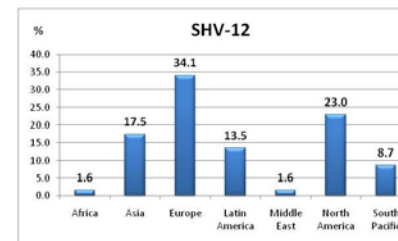
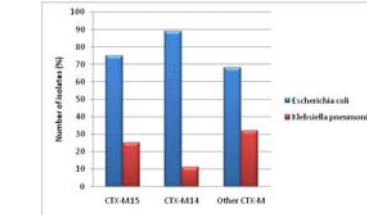


Table 1: Distribution of ESBL genes (%) by species

Species/ESBL	CTX-M	SHV	TEM	SHV+CTX-M	SHV+TEM	TEM+CTX-M	SHV+TEM+CTX-M
<i>E. coli</i>	91.7	5.9	0.7	1.4	-	0.4	-
<i>K. pneumoniae</i>	60.1	32	0.6	5.7	-	0.9	0.6
<i>K. oxytoca</i>	75	25	-	-	-	-	-
<i>E. cloacae</i>	46.7	46.7	-	3.3	3.3	-	-
<i>E. aerogenes</i>	75	25	-	-	-	-	-
<i>P. mirabilis</i>	90.9	-	9.1	-	-	-	-

Figure 6: Distribution of the most common CTX-M genes among E. coli and K. pneumoniae isolates



-CTX-M15 (66%)  
-CTX-M14 (13%)

Figure 7: Worldwide distribution of CTX-M15 and CTX-M14 genes among 1,119 ESBL-producing isolates

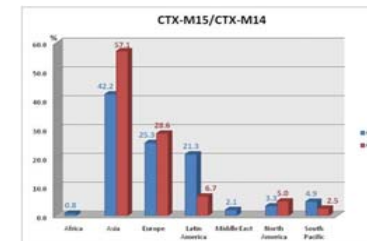
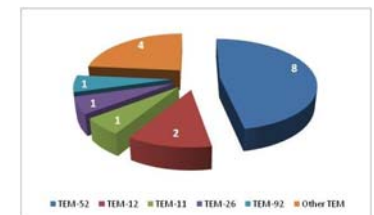
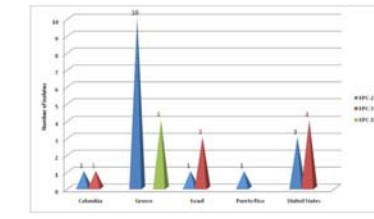


Figure 7: Worldwide distribution of TEM genes (17 isolates)



-TEM52 (47%)

Figure 9: Worldwide distribution of KPC genes among 28 KPC-positive isolates



## Conclusions

Check-Points array combined with PCR-sequencing may become a key method in large epidemiological studies and infection control follow-up

Check-Points array is truly a promising tool for the rapid detection of ESBLs but also AmpC and carbapenemases, especially with the recent emergence of NDM-1.

CTX-M type ESBLs are currently the most common enzymes while the SHV-type is less common and the TEM-type is becoming rare

The clinically relevant ESBLs remain CTX-M15 and CTX-M14 but also SHV-12, SHV-5 and SHV-2.

The most common KPC genes are KPC-2 and KPC-3

## References

- Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S19, 2009. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
- Bonnet, R. 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1-14.
- Cuzon, G., T. Naas, and P. Nordmann. [KPC carbapenemases: what issue in clinical microbiology?]. Pathol Biol (Paris) 58:39-45.
- Jones, C. H., A. Ruzin, M. Tuckman, M. A. Visalli, P. J. Petersen, and P. A. Bradford. 2009. Pyrosequencing using the single-nucleotide polymorphism protocol for rapid determination of TEM- and SHV-type extended-spectrum beta-lactamases in clinical isolates and identification of the novel beta-lactamase genes blaSHV-48, blaSHV-105, and blaTEM-155. Antimicrob Agents Chemother 53:977-86.
- Mulvey, M. R., E. Bryce, D. Boyd, M. O'Neil, S. Christianison, A. E. Simer, and S. Paton. 2004. Ambler class A extended-spectrum beta-lactamase polymorphism-PCR. Antimicrob Agents Chemother 48:1204-14.
- Nuesch-Inderbinen, M. T., H. Hachler, and F. H. Kayser. 1996. Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. Eur J Clin Microbiol Infect Dis 15:398-402.
- Pfout, J. D., and K. B. Laupland. 2008. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8:159-66.
- Speldoren, V., B. Heym, R. Labia, and M. H. Nicolas-Chanoine. 1998. Discriminatory detection of inhibitor-resistant beta-lactamases in Escherichia coli by single-strand conformation polymorphism-PCR. Antimicrob Agents Chemother 42:879-84.
- Woodford, N., E. J. Fagan, and M. J. Ellington. 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. J Antimicrob Chemother 57:154-5.