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# Use of Check-points Micro array for Beta-lactamase Detection Among Enterobacteriaceae Bacteremia Isolates from USA Hospitals

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## AMENDED ABSTRACT

**Background**: Molecular detection of β-lactamases (BL) is a laborious task that limits its use in clinical laboratories and other settings. Check-Points molecular assays use PCR and micro array to detect several clinically relevant BLs, including ESBLs and carbapenemases. We evaluated the Check-MDR CT101 kit (CP) to screen ESBL-phenotype-positive Enterobacteriaceae strains from USA medical centers and compared to reference molecular methods.

**Methods**: Among 185 (8.0% overall of strains collected) isolates that met the CLSI ESBL MIC criteria, 109 *Klebsiella* spp. and *E. coli* (EC) were selected for analysis. Isolates were recovered from blood cultures from 25 USA hospitals (19 states) during 2010 and susceptibility tested by CLSI methods. PCR and sequencing for genes encoding TEM, SHV, CTX-M, PER, VEB, GES, PSE, OXA-2, OXA-10, OXA-18/-45, OXA-1/30 and plasmidic (p) AmpCs were performed. *K. oxytoca* (KOX) strains were also tested for $bla_{OXY}$. CP was used according to the manufacturer's instructions. Discrepant strains were repeat tested and/or submitted to isoelectric focusing (IEF).

**Results**: CP showed that 53 strains carried $bla_{CTX-M}$ group 1 genes and 15 carried $bla_{CTX-M}$ group 9. pAmpCs genes $bla_{CMY}$ and $bla_{FOX}$ were detected in 11 and 2 strains, respectively. KPC variants were observed in 6 strains, one carbapenem-susceptible *K. pneumoniae* (imipenem MIC, 0.5 µg/mL). SHV ESBL genes were detected in 16 strains and 10 also carried a wildtype (WT) variety of this gene. TEM WT genes were observed among 56 strains. CP was able to detect 33 different enzyme arrays (>1 enzyme). Concordance between CP and reference PCR/sequencing for genes tested in both methods was observed for 105 strains (96.3%). Four strains showing discrepancies had WT TEM resultsin one method and not the other, confirmed by IEF. Among 32 strains genes that are not targeted by CP were found (OXA ESBLs, PER, PSE, OXY); however in 30 cases another ESBL or carbapenemase was detected in the strain, generating only two false-negatives. Two KOX were negative by CP, but carried $bla_{OXY}$.

**Conclusions**: Check-points is a reliable, robust and user friendly assay for BL detection. Limitations noted were related to genes that are not included in the platform and very few false-positive TEM WT signals. This method could potentially be used more widely in clinical laboratories.

## INTRODUCTION

Several types of acquired extended spectrum β-lactamases (ESBLs) conferring elevated MIC values to penicillins, cephalosporins and aztreonam have been described among Enterobacteriaceae. CTX-M, TEM and SHV variants are the most numerous and prevalent; however, a variety of other enzymes such as VEB, GES/IBC, PER, BEL, GES etc. have been detected among these organism. Additionally, plasmid-mediated cephalosporinase enzymes have also emerged through the mobilization of chromosomal genes of inducible AmpC β-lactamases onto plasmids. When transferred into other organisms such as *Escherichia coli* and *Klebsiella pneumoniae*, these cephalosporinases have similar substrate profiles to the parent chromosomal enzymes but differ in having constitutively expressed enzyme activity.

Moreover, the presence of multiple β-lactamases in a single cephalosporin-resistant isolate has been recognized as an alarming fact since early detection of these enzymes. Although strains reported to produce two to four β-lactamases were commonly characterized, the recent detection of a single *K. pneumoniae* strain carrying up to eight β-lactamase encoding genes, including a KPC carbapenemase and an inhibitor-resistant TEM variant, emphasizes the ability of these organisms to accumulate multiple resistance determinants.

Detection of β-lactamases is a time consuming and difficult task that requires specialized technical know-how and personnel due to the many enzyme families and variants that have been described. Standard PCR and gene sequencing is still the most widely used technique and other molecular detection methods for β-lactamase-encoding genes have been proposed, but none seems suitable for use in routine clinical microbiology laboratories. We evaluated the Check-MDR CT101 kit (Check-points, Wageningen, The Netherlands), a micro array based method for detection of CTX-M, TEM, SHV, KPC, NDM-1 and plasmid mediated AmpC, compared to conventional PCR/sequencing methods for a contemporary collection of *E. coli* and *Klebsiella* spp. displaying the CLSI ESBL epidemiological screening criteria.

## MATERIALS AND METHODS

Bacterial isolates. Among 185 Enterobacteriaceae susceptibility tested using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, M07-A9) that displayed the CLSI epidemiological screening criteria, 109 *E. coli* and *Klebsiella* spp. were selected for screening by reference PCR/sequencing and microarray methods for detection of ESBLs and other plasmid-mediated enzymes.

PCR detection and sequencing of β-lactamase genes. PCR screening was performed for $bla_{TEM}$, $bla_{SHV}$ (singleplex reactions), $bla_{CTX-M}$, $bla_{GES}$, $bla_{VEB}$, $bla_{PER}$, $bla_{PSE}$, $bla_{BEL}$, and oxacillinases with ESBL spectrum ($bla_{OXA-2}$-, $bla_{OXA-10}$- and $bla_{OXA-30}$-group, $bla_{OXA-18}$ and $bla_{OXA-45}$) in a combination of multiplex reactions. Additionally, $bla_{CMY-1-41}$, $bla_{CMY-43-44}$, $bla_{CMY-49}$, $bla_{FOX-1-7}$, $bla_{ACC-1-4}$, $bla_{ACT-1-7}$, $bla_{DHA-1-3}$, $bla_{LAT-1}$, $bla_{MIR-1-5}$, $bla_{MOM-1-7}$ were also amplified in a multiplex reaction. Isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/mL) were screened for production of the following carbapenemases: $bla_{IMP}$, $bla_{VIM}$, $bla_{SPM-1}$, $bla_{KPC}$, $bla_{SME}$, $bla_{IMI}$, $bla_{NMC-A}$, $bla_{GES}$ and $bla_{OXA-48}$ by PCR. Amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Commercial micro array. The Check-MDR CT101 kit was used according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M Groups 1, 2, 8+25 and 9, TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM-1. DNA from isolates showing discordant results compared to PCR/sequencing were sent to manufacturer and repeated.

Isoelectric focusing (IEF). Protein preparations were obtained with BugBuster® (Novagen, Darmstadt, Germany) and submitted to IEF gels to determine the presence of β-lactamases in isolates showing discrepant results between PCR/sequencing and the Check-points microarray. Gels were stained using nitrocefin and isoelectric point (pI) was determined by linear regression using a combination of known β-lactamases and a pre-stained protein marker.

## RESULTS

- By PCR and sequencing methods, isolates carried a variety of genes and a total of 33 β-lactamase arrays (>1 enzyme) were observed (Table 1). Two *E. coli* isolates carried no detectable β-lactamase encoding genes.
- Check-MDR CT101 displayed a good correlation with PCR/sequencing for the genes tested by both methods, with only four (4) discrepant results (Table 2). Two isolates displayed TEM WT results on Check-MDR 101, but not by PCR/sequencing, whereas two (2) isolates displayed negative TEM WT results and $bla_{TEM-1}$ was repeatedly detected by PCR/sequencing. These results were confirmed by IEF.
- A total of 37 isolates (Table 2) had genes not detected by Check-MDR CT101, but in only three instances the overall result for ESBL presence was discordant between the two methods. Two *K. oxytoca* strains carried derepressed $bla_{OXY}$ that is not included on the micro array and one *K. pneumoniae* carried genes encoding OXA-2, SHV-1 and TEM-1 and the micro array only detected TEM and SHV WT.
- Sensitivity and specificity of Check-MDR CT101 compared to PCR/sequencing for the tests included in both methodologies was very high: 0.98 and 0.99, respectively.
- Most prevalent enzymes among *E. coli* and *Klebsiella* spp. collected in USA hospitals during 2010 according to Check-MDR CT101 were TEM WT (56 occurrences; 28%) followed by CTX-M Group 1 (53 occurrences; 27%), SHV WT (40; 20%), SHV ESBL (16; 8%), CTX-M Group 9 (15; 8%), CMYII (11; 5%), KPC (6; 3%) and FOX (2; 1%; Figure 1).

**Table 1**. Enzymes arrays and β-lactamases detected by PCR/sequencing among 109 Enterobacteriaceae strains tested. Two isolates did not yield results for the β-lactamases tested by both methods.

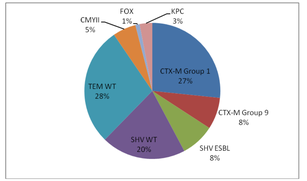
| Enzyme arrays detected[a] | No. of isolates | Enzyme arrays detected[a] | No. of isolates |
|---|---|---|---|
| CTX-M-15-like+OXA-1/30 | 13 | CMY-2+SHV-33 | 1 |
| CTX-M-15-like | 9 | CMY-like+TEM-1 | 1 |
| CTX-M-15-like+TEM-1 | 7 | CTX-M-14+SHV-like+TEM-1 | 1 |
| CTX-M-15-like+OXA-1/30+TEM-1 | 6 | CTX-M-15-like+KPC-2+SHV-1+TEM-1 | 1 |
| CTX-M-15-like+SHV-1+TEM-1 | 5 | CTX-M-15-like+KPC-3+OXA-1/30+SHV-11+TEM-1 | 1 |
| CMY-2 | 4 | CTX-M-15-like+OXA-1/30+PSE-like | 1 |
| CMY-2+TEM-1 | 4 | CTX-M-15-like+OXA-1/30+SHV-1 | 1 |
| CTX-M-14+TEM-1 | 4 | CTX-M-15-like+OXA-1/30+SHV-1+TEM-1 | 1 |
| TEM-1 | 4 | CTX-M-27+OXA-1/30 | 1 |
| CTX-M-15-like+OXA-1/30+SHV-11+TEM-1 | 3 | FOX-5+PSE-like+SHV-11 | 1 |
| CTX-M-15-like+SHV-1 | 3 | FOX-5+SHV-11 | 1 |
| KPC-3+SHV-11+TEM-1 | 3 | KPC-2+OXA-2-like+OXY-like | 1 |
| SHV-11 | 3 | OXA-1/30+SHV-7+TEM-1 | 1 |
| SHV-12+TEM-1 | 3 | OXA-2-like+SHV-1+TEM-1 | 1 |
| CTX-M-14 | 2 | OXA-9+SHV-7+TEM-1 | 1 |
| CTX-M-14+SHV-11+TEM-1 | 2 | SHV-1+SHV-12+TEM-1 | 1 |
| CTX-M-15-like+SHV-11 | 2 | SHV-1+TEM-1 | 1 |
| CTX-M-27 | 2 | SHV-11+SHV-5+TEM-1 | 1 |
| CTX-M-27+SHV-11+TEM-1 | 2 | SHV-2 | 1 |
| OXY-like | 2 | SHV-30 | 1 |
| SHV-like | 2 | SHV-like+TEM-1 | 1 |
| CMY-2+CTX-M-14 | 1 | | |

a. Enzymes were named "-like" when sequencing did not exhibit enough coverage to differentiate among variants or when new enzymes were detected.

**Table 2**. Isolates displaying discrepancies (n=37 isolates) between reference PCR/sequencing results and Check-MDR CT101 results (underlined for tests not included in the Check-MDR and red underlined for tests included in the assay). Bolded rows represent the isolates that were negative for ESBL production by the Check-MDR, but were positive for the reference testing.

| | | | | Check-MDR CT101 kit results | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organism | City | State | PCR/Sequencing Results | CTX-M Group 1 | CTX-M Group 2 | CTX-M Groups 8+25 | CTX-M Group 9 | SHV ESBL | SHV WT | TEM ESBL | TEM WT | ACC | ACT/MIR | CMYII | DHA | FOX | KPC | NDM-1 |
| *E. coli* | Rochester | NY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Lexington | KY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | New York | NY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Aurora | CO | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | New York | NY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | New Brunswick | NJ | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | New York | NY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Charlottesville | VA | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Rochester | NY | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Charlottesville | VA | CTX-M-15-like+OXA-1/30 | pos | | | | | | | | | | | | | | |
| *E. coli* | Omaha | NE | CTX-M-15-like+OXA-1/30+PSE-like | pos | | | | | | | | | | | | | | |
| *E. coli* | New York | NY | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Des Moines | IA | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Akron | OH | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Detroit | MI | CTX-M-15-like+OXA-1/30+TEM-1 | pos | | | | | | | pos | | | | | | | |
| *E. coli* | New York | NY | CTX-M-27+OXA-1/30 | | | | | pos | | | | | | | | | | |
| *E. coli* | Charlottesville | VA | OXA-9+SHV-7+TEM-1 | | | | | | pos | | pos | | | | | | | |
| *E. coli* | Louisville | KY | CTX-M-15-like | pos | | | | | | | pos | | | | | | | |
| *E. coli* | Pensacola | FL | CTX-M-15-like | pos | | | | | | | pos | | | | | | | |
| ***K. oxytoca*** | **New Brunswick** | **NJ** | **OXY-like** | | | | | | | | | | | | | | | |
| ***K. oxytoca*** | **New York** | **NY** | **OXY-like** | | | | | | | | | | | | | | | |
| *K. oxytoca* | Charlottesville | VA | KPC-2+OXA-2-like+OXY-like | | | | | | | | | | | | | | pos | |
| *K. pneumoniae* | New York | NY | CTX-M-15-like+KPC-3+OXA-1/30+SHV-11+TEM-1 | pos | | | | | pos | | pos | | | | | | pos | |
| *K. pneumoniae* | New York | NY | CTX-M-15-like+OXA-1/30+SHV-1 | pos | | | | | pos | | | | | | | | | |
| *K. pneumoniae* | Indianapolis | IN | CTX-M-15-like+OXA-1/30+SHV-1+TEM-1 | pos | | | | | pos | | pos | | | | | | | |
| *K. pneumoniae* | New York | NY | CTX-M-15-like+OXA-1/30+SHV-11+TEM-1 | pos | | | | | pos | | pos | | | | | | | |
| *K. pneumoniae* | Akron | OH | CTX-M-15-like+OXA-1/30+SHV-11+TEM-1 | pos | | | | | pos | | pos | | | | | | | |
| *K. pneumoniae* | Detroit | MI | CTX-M-15-like+SHV-11 | pos | | | | | pos | | pos | | | | | | | |
| *K. pneumoniae* | Lexington | KY | CTX-M-15-like+OXA-1/30+SHV-11+TEM-1 | pos | | | | | pos | | | | | | | | | |
| *K. pneumoniae* | Louisville | KY | FOX-5+PSE-like+SHV-11 | | | | | | pos | | | | | | | pos | | |
| *K. pneumoniae* | New York | NY | OXA-1/30+SHV-7+TEM-1 | | | | | pos | pos | | pos | | | | | | | |
| ***K. pneumoniae*** | **New York** | **NY** | **OXA-2-like+SHV-1+TEM-1** | | | | | **pos** | **pos** | | **pos** | | | | | | | |

**Figure 1**. Most common types of ESBL, pAmpC and carbapenemase enzymes detected by the Check-MDR CT101 kit.




## CONCLUSIONS

- Check-MDR CT101 kit displayed high sensitivity and specificity compared to reference PCR/sequencing for detection of prevalent β-lactamases is USA *E. coli* and *Klebsiella* spp. isolates. This method is easy to use and seems suitable for clinical microbiology laboratory applications.
- Among ESBLs, CTX-M-15 and CTX-M-14 with or without other enzymes are becoming very prevalent in USA hospitals. CMY variants and SHV enzymes with extended spectrum were also common.

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