C-607

Evaluation of a PCR-Microarray Assay (Check-MDR CT101, Wageningen, The Netherlands) for the Detection of Carbapenemase-Producing *Enterobacteriaceae*

A. A. Roberts[1], T. Tekle[1], P. D. Stamper[2], K. C. Carroll[1,2]

[1]The Johns Hopkins Hospital Microbiology Laboratory, Baltimore, MD, [2] Department of Pathology The Johns Hopkins Univ. Sch. of Med., Baltimore, MD.

UPDATED ABSTRACT

Background: The increasing prevalence of antimicrobial resistance in gram-negative bacteria highlights the need for rapid and accurate detection methods. The Check-MDR CT101 Assay (Check-Points, Wageningen, The Netherlands) is a microarray-based diagnostic assay that identifies the presence of TEM, SHV, CTX-M, KPC, NDM-1 and AmpC genes. The assay employs highly specific DNA markers on carefully selected SNPs on an array which allows for the detection and distinction between resistance determinants. In this study, we tested 111 well characterized multi-drug resistant *Enterobacteriaceae* using the Check-MDR assay and compared the results to both phenotypic and genotypic methods in use in our clinical laboratory.

Methods: During the study period, 111 clinical gram negative isolates recovered from a variety of patient cultures were tested using the Check-MDR CT101 protocol which includes three steps (1) DNA ligation, (2) DNA amplification, and (3) array detection. All clinical isolates had been previously characterized by Modified Hodge Testing using CLSI guidelines and an in-house multiplex real-time PCR assay for KPC genes 1-7.

Results: The overall agreement was 98.2% for the two genotypic assays and 90.1% between the Modified Hodge Test and the Check-MDR CT 101 assay. Forty-seven isolates were Modified-Hodge positive, KPC PCR positive, and Check-MDR CT101 positive. Fifty-three isolates were Modified-Hodge negative, KPC PCR negative, and Check-MDR CT 101 negative. Seven isolates were Modified-Hodge positive, KPC PCR negative, and Check-MDR negative. Two isolates were Modified-Hodge negative, KPC PCR positive, and Check-MDR positive. Two isolates were Modified-Hodge negative, KPC PCR negative, and Check-MDR CT101 positive.

Conclusion: The Check-MDR CT 101 method was more accurate than the Modified Hodge Test and correlated well with the in-house multiplex PCR method for detection of carbapenemase-producing gram negative rods. This assay has the advantage of simultaneously detecting other resistance determinants not evaluated in this study. However, the assay in its current form requires a full working day to perform and is somewhat labor intensive.

INTRODUCTION

Carbapenemase-producing *Enterobacteriaceae* are found worldwide. *Klebsiella pneumoniae* carbapenemase (KPC) is the most common type in the United States among clinically important gram-negative bacteria. KPC enzymes are predominantly found on transferable plasmids in *Klebsiella pneumoniae* but occur in other *Enterobacteriaceae* as well. These organisms are resistant to almost all antimicrobials which challenges healthcare providers and places pressure on laboratories to produce rapid and accurate results. At the JHH, the prevalence of KPC-producing organisms was determined to be 36% of isolates tested during an 18 month period (July 2008-March 2009). [1]

GOAL OF STUDY

To evaluate the Check-MDR CT101 Assay to the current phenotypic and genotypic laboratory methods used to identify and characterize Carbapenemase-producing *Enterobacteriaceae.*

REFERENCE METHODS

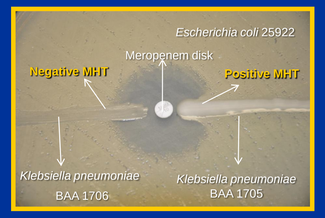
Isolate Selection

- NMIC/ID -132 Panel for BD-Phoenix Automated Microbiology System (BD Diagnostics, Spark, MD) was used for identification and susceptibilities.
- *Enterobacteriaceae* except *Proteus spp*., *Providencia spp*., and *Morganella spp*. were evaluated.
- CLSI guidelines were used to determine if isolates were KPC screen Positive (ertapenem 2 µg/ml **or** imipenem 2 to 4 µg/ml **or** meropenem 2 to 4 µg/ml **and** resistant to one or more agents in cephalosporin subclass III).

Modified Hodge Testing (MHT)

- A bacterial lawn using a 1:10 dilution of a 0.5 McFarland of *Escherichia coli* 25922 was made on a Mueller-Hinton agar plate.
- A clinical isolate was then swabbed from a meropenem disk on the lawn plate.
- After 16-24 h of incubation, an indentation will appear for a positive MHT and there will be no indentation for negative MHT.

Figure 1: Modified Hodge Test



KPC PCR

- All isolates were extracted using the Manual QiAmp DNA Mini Kit for extraction of whole-cell DNA.
- Tested on the Cepheid Smart Cycler.
- The KPC PCR detects bla_{KPC} 1-7 genes[2].

TEST METHOD

Check –MDR CT101

- All isolates were extracted using the Manual QiAmp DNA Mini Kit for extraction of whole-cell DNA.
- After PCR on the PE 9600, the amplified product was analyzed by the Check-MDR CT101 array using the multiplex ligation detection. One probe consists of a short target-specific sequence and primer for PCR while the other is a longer probe and primer that is target-specific with a ZIP code on the microarray. The two probes are ligated to each other when hybridized to a specific target sequence and subsequently amplified (See Figure 2).
- Positive hybridization is shown using a biotin label incorporated within the primers detected in the DNA microarray. There are 27 resistance markers and 19 control markers on each microarray that is only recognized by one probe pair. Three samples are mixed with a different probe mixture allowing hybridization in different locations on the microarray (See Figure 3).
- Microarrays were read on a single channel reader connected to a standard computer. The data were analyzed by custom software that translated the microarray data along with controls demonstrating each step in the procedure (See Figure 4).

Figure 2: Check-MDR CT101 Assay

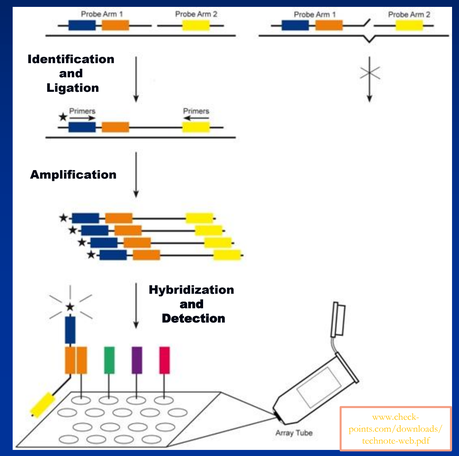
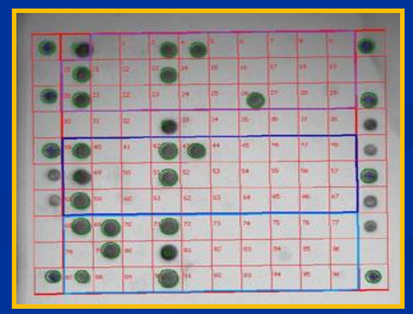


Figure 3: Check-MDR CT101 Array Layout

Figure 4: Computer Analysis of an Array



RESULTS

- One Hundred eleven isolates were "screen positive" by the BD-Phoenix Automated Microbiology System (BD Diagnostics, Spark, MD). Using the Modified Hodge Test 48.6% (54/111) of the *Enterobacteriaceae* were positive and phenotypically resistant to carbapenems (See Figure 5).
- Comparison of the Check-point assay to the KPC PCR resulted in 98.2% (109/111) agreement. The sensitivity, specificity, and positive and negative predictive values were 100% (49/49), 96.8% (60/62), 96.1% (49/51), and 100% (60/60), respectively (See Table 1).
- Two isolates testing positive by the Check-MDR CT101 but negative with the KPC PCR were *Klebsiella pneumonia*e. These two isolates were negative by the phenotypic test and both were deemed as true negatives by KPC PCR .

Figure 5. Phenotypic Characterization of Isolates (n=111)

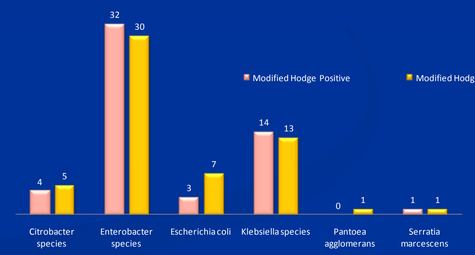


Table 1. Comparison of Check-MDR CT101 to KPC PCR

	KPC PCR		
Check-MDR	**Positive**	**Negative**	**Total**
Positive	49	2	51
Negative	0	60	60
Total	49	62	111

SUMMARY

- The discrepancy among the two isolates that were positive for Check-MDR CT101 but negative for the KPC PCR and MHT may be due to contamination of the array. The isolates could not be retested due to lack of materials.
- Contamination is of high concern when performing the testing and it is recommended to use separate rooms when completing each of the three steps.
- When compared to PCR and the Modified Hodge test, the Check-MDR CT101 performed very well for the detection of KPC enzymes
- Check-MDR CT101 has instant automated analysis and can be implemented in routine clinical testing.

REFERENCES

1. Tekle, T. P.D. Stamper, L. Maragakis, K.C. Carroll. Johns Hopkins Medical Institutions, Baltimore, MD.Prevalence of KPC Producing Enterobacteriacea in a Teriary Care Hospital Settingin a Low Prevalence Geographic Region. Abstract #3119 49th ICAAC meeting, San Francisco,CA.
2. Hindiyeh, M., et al. 2008. Rapid Detection of *bla*$_{KPC}$ Carbapenemase Genes by Real-Time PCR. *J. Clin. Microbiol.*; Vol. 46, p. 2879-2883.

We would like to thank Check-Points for their Material Support.