548

Ten Minute, Reagent-Free identification of Bacteria Containing Resistance Genes Using a Rapid Intrinsic Fluorescence Method

R. Rozen-Sadowsky[1], A. Shinderman[1], D. Gohman[1], D. Shimonov[1], Y. Gluckman[1], G. Ingber[1];
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548

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Abstract

Background: POCARED™ P1000™ system (POCARED Diagnostics Ltd.) is an automated rapid platform that employs intrinsic fluorescence, optical data analysis and artificial intelligence methods to analyze multi-dimensional optical characteristics of microorganisms. The purpose of this study was to evaluate the ability of POCARED™ P-1000™ system to differentiate isolates of commonly encountered bacteria that contain specific resistance genes from similar isolates that do not harbor the gene.

Methods:

Strains evaluated containing antibiotic resistant genes included: A*cinetobacter baumannii* (AB) with *blaOXA* (n=2), *Escherichia coli* (EC) with *blaNDM* (n=3) , *Klebsiella pneumoniae* (KP) with *blaNDM* (n=5), EC with *blaKPC* (n=1), KP with *blaKPC* (n=5), *Enterococcus faecalis* (EF) with *vanB* (n=2) and *Staphylococcus aureus* (SA)with *mecA* (n=4). Control strains without resistance genes included: 1 each of AB, EC, EF, KP and SA. All isolates were obtained from ATCC. Bacterial suspensions containing 10^8 CFU/ml were analyzed using the POCARED™ P1000™ system which measures Excitation Emission Matrix (EEM) of the sample. The EEMs were processed using chemometric technique and for each isolate tested 30 times a prediction was generated. The accuracy for the prediction was calculated as the total number of correct predications divided by the total number of measurements.

Results: The accuracy for detecting isolates containing a specific resistance gene was 98%, 96%, 95%, 97% and 95% for AB, EC, EF, KP and SA, respectively. The accuracy for differentiating bacteria containing *blaKPC* or *blaNDM* in EC and KP was 99% and 98%, respectively.

Conclusion: Using chemometric analysis of EEMs which is the basis of POCARED™ P1000™ system we were able to differentiate bacteria with a resistance gene from bacteria without a resistance gene. In addition, we were able to differentiate between bacteria with different resistance genes

Introduction:

As bacteria resistant to antibiotics is increasing the need for real-time detection and identification becomes crucial[1,2]. Different diseases are associated with different bacterial types. The diagnosis of these infections depends largely on time and labor consuming (48-72 hours) classical microbiological and biochemical methods for species identification and susceptibility testing methods (disc diffusion and broth micro-dilution).[1] During that time patients are treated by empirical antibiotic treatment (with wide spectrum antibiotics) which can lead to both side effects and increased bacterial resistance to antibiotics[1,2]. Recently, polymerase chain reaction (PCR) has been used for rapid detection of specific resistant genes in different bacteria[1,2].In addition, multiplex PCR assays have been developed to allow simultaneous species-specific identification of several bacteria with several relevant antibiotic resistant genes[3,4]. These genotypic rapid detection assays allow direct detection of both bacterial type and resistance genes from positive clinical specimen[1,3] and contribute to a reduction of empirical antibiotic treatment, but still need a few hours for detection and are labor and regent consuming[1-4].

POCARED™ P-1000™ system (POCARED Diagnostics Ltd.) (Figure 1) is an automated, rapid, reagentless platform that employs intrinsic fluorescence, optical data analysis and artificial intelligence methods to analyze multi-dimensional optical characteristics of microorganisms. An advance fluorometer utilizes a UV light source to excite the microorganisms in the sample to create fluorescent energy (autofluorescence). Neither reagents nor tagging is required to generate the autofluorescence signal. The intensity and spectral content of the autofluorescence signal is captured by a photodetector. The captured signal is converted into a mathematical model that is used to provide the results.

In this study we've determined the ability of POCARED™ P-1000™ to differentiate isolates of commonly encountered bacteria that contain specific resistance genes from similar isolates that do not harbor the gene.

Methods:

1. **Bacteria**

21 Antibiotic resistant strains and 5 control strains were purchased from the American Type Culture Collection (ATCC) and included as shown below:

Organism	Resistance Gene	ATCC strains
Acinetobacter baumannii	OXA	BAA-2093 BAA-1798
	Control	BDPOC-08
E. coli	KPC	BAA-2340
	NDM	BAA-2452 BAA-2471 BAA-2469
	Control	25922
Enterococcus faecalis	Van B	51575 BAA-2365
	Control	29212
Klebsiella pneumoniae	KPC	BAA-1898 BAA-1902 BAA-1903 BAA-1904 BAA-2078
	NDM	BAA-2146 BAA-2470 BAA-2472 BAA-2473
	Control	13883
Staphylococcus aureus	mecA	BAA-2312 BAA-2313 BAA-2094 BAA-2422
	Control	25923

2. **Bacteria sample preparation and plating**

- An aliquot from stock cultures of each test organism frozen at -80°C was inoculated onto blood agar plates (Hy Labs, Israel)
- After overnight incubation, cell paste from several colonies was inoculated into PBS (Sigma-Aldrich, USA) to obtain a suspension containing 10^8 CFU/ml.
- Each suspension was further diluted to 10^4 and 10^3 CFU/ ml and these dilutions were subcultured to BAPs to check for purity.
- 30 replicates suspensions were prepared for each isolate

3. **P-1000™ process and analyzing of bacteria**

- The bacterial suspensions containing 10^8 CFU/ml were analyzed using the POCARED™ P-1000™ system which measures the Excitation Emission Matrix (EEM) of the sample.
- The EEMs were processed using a Partial Least Squares Discriminant Analysis (PLS-DA) [5]
- The accuracy for the prediction was calculated as follows

Results:

1. The POCARED™ P-1000™ system measures the Excitation Emission Matrix of the sample. A total of 1176 samples were measured and PLS- DA model was constructed

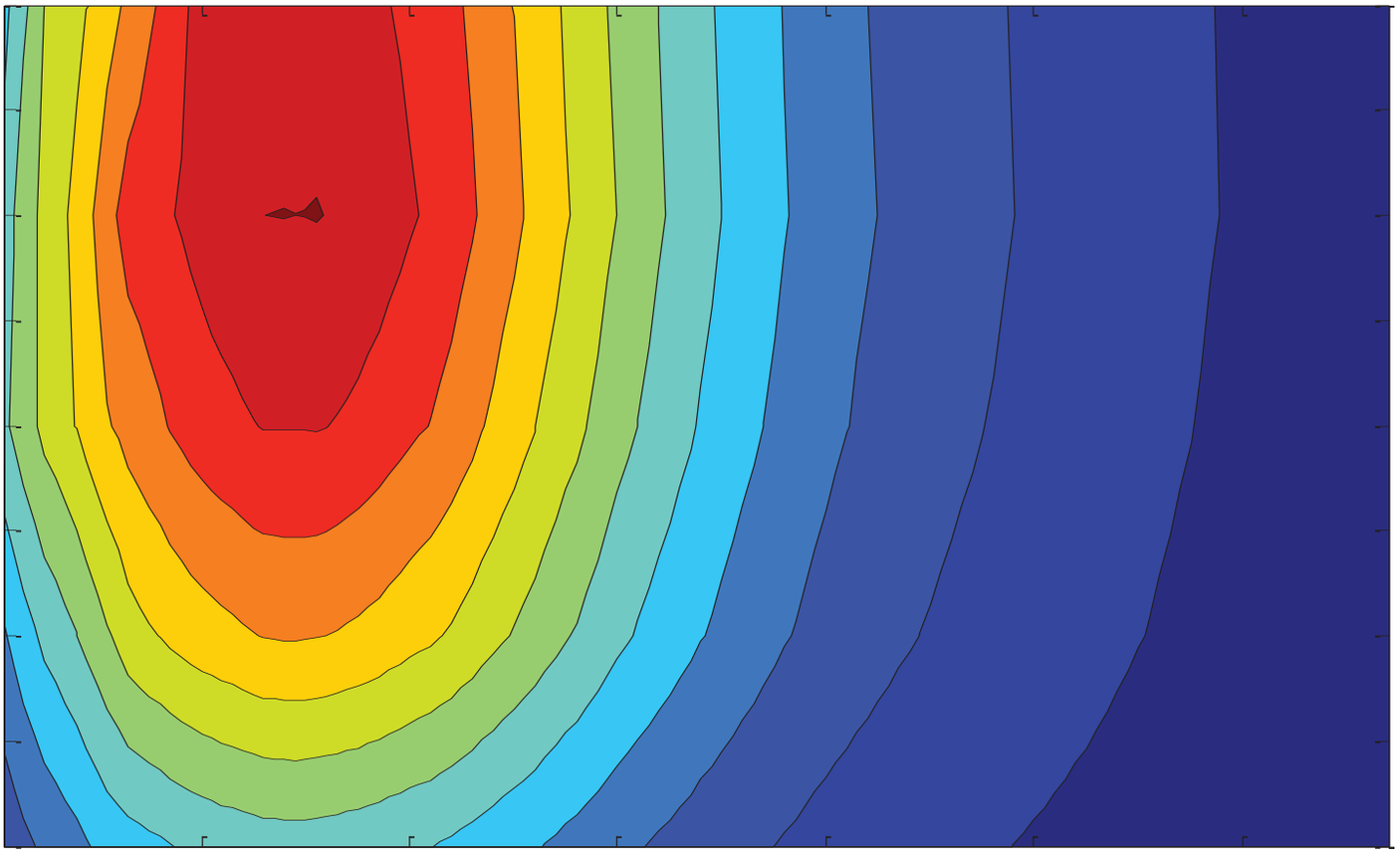

Figure 2: EEM measured by The POCARED™ P-1000™ system

2. Venetian blinds w/ 10 splits and 1 samples per split was used for cross validation of the gene existence model (comparing to the known information on the existence of a resistance gene in that isolate as appears in the ATCC records for that isolate)

		Actual	
		OXA	Control
Predicted	OXA	58	0
	Control	2	55

Confusion matrix for *Acinetobacter baumannii*

		Actual	
		NDM or KPC	Control
Predicted	NDM or KPC	162	4
	Control	10	87

Confusion matrix for *E. coli*

		Actual	
		Van B	Control
Predicted	Van B	57	3
	Control	3	57

Confusion matrix for *Enterococcus faecalis*

		Actual	
		NDM or KPC	Control
Predicted	NDM or KPC	275	1
	Control	13	149

Confusion matrix for *Klebsiella pneumoniae*

		Actual	
		MecA	Control
Predicted	MecA	115	7
	Control	5	113

Confusion matrix for *Staphylococcus aureus*

3. The accuracy for detecting isolates containing a specific resistance gene was 98%, 96%, 95%, 97% and 95% for AB, EC, EF, KP and SA, respectively.

4. Venetian blinds w/ 10 splits and 1 samples per split was used for cross validation of the specific gene identification model of the EC and KP that had isolates with different resistance gene (comparing to the known information on the existence of that specific resistance gene in that isolate as appears in the ATCC records for that isolate)

		Actual	
		NDM	KPC
Predicted	NDM	85	2
	KPC	0	85

Confusion matrix for gene discrimination in *E.Coli*

		Actual	
		NDM	KPC
Predicted	NDM	138	3
	KPC	3	144

Confusion matrix for specific gene discrimination in *Klebsiella pneumoniae*

5. The accuracy for differentiating bacteria containing *blaKPC* from *blaNDM* in EC and KP was 99% and 98%, respectively.

Conclusions:

1. Using chemometric analysis of EEMs which is the basis of POCARED™ P-1000™ system, for each bacterial species, we were able to differentiate isolates that contained a resistance gene from isolates of the same species that did not contain the resistance gene.
2. In addition, we were able to differentiate between bacteria with different resistance genes (*blaKPC* or *blaNDM*) in EC and KP.

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Figure 1: POCARED™ P-1000™ Platform