Comparing Culture Based Methods To A Novel PCR-based Multiplex Assay To Detect Carbapenemase Genes In Perirectal Swabs

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Background

Multidrug resistant (MDR) Gram negative bacteria are among the greatest therapeutic challenges facing world health. Methods for screening patients for MDR GNB include culture of perianal swab samples on selective agar plates followed by antimicrobial susceptibility testing.

Screening for carbapenemase producing *Enterobacteriaceae* (CPE) is usually done by supplementing MacConkey Agar with imipenem before inoculation of the test strain[1]. The CDC recommends an ertapenem-enriching step on ertapenem-containing trypticase-soy broth followed by inoculation into MacConkey Agar[2]. Different media have been developed to try to optimize detection while obtaining results in a shorter time span.

Other media used to detect CPE include SUPERCARBA agar and chromogenic media. The SUPERCARBA agar is a Drigalski-lactose media supplemented with cloxacillin (250 µg/mL), zinc (70 µg/mL), and ertapenem (0.5 mg/L)[3]. These media have been compared regarding their limit of detection of CRE at different inocula and when used for stool screening[1,4–8]. Some of these agar media have improved ability to detect KPC and have decreased sensitivity for mechanisms based on other enzymes, particularly OXA-48[5].

Method	Sensitivity(%)	Specificity (%)
ChromID KPC	84.9	88.7
MacConkey/Imipenem	84.9	94.3
CDC Protocol	98.8	80
chromID CARBA	92-100	91-98
***Brilliance* CRE**	59	34

Screening molecular techniques such as Real Time PCR have been used with the advantage of more rapid results, increased sensitivity, and increased specificity[9–11]. Sensitivity and specificity are higher than for culture-based methods.

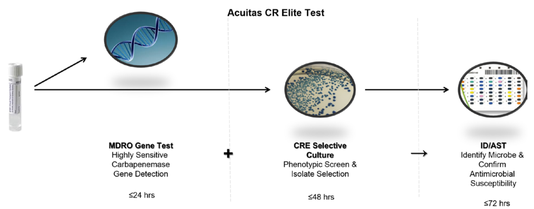
Target	Methodology	Sensitivity	Specificity	Limit of detection (CFU/mL)	N
VIM, IMP, KPC, OXA-48, NDM-1 [12]	PCR (Hyplex SuperBug ID)	98.0%	98.6%	Not calculated	236
KPC, NDM-1 [13]	qPCR	100%	100%	KPC: 10^4-10^5 CFU/mL NDM: 10^3 CFU/mL	46/80*
VIM, OXA-48, NDM, KPC [14]	qPCR(Check-MDR Carba)	100%	100%	10^3-10^5 CFU/mL	25
KPC, NDM, VIM [15]	qPCR(Xpert MDRO)	100%	99%	< 3 X 10^2 CFU/mL	328

Objectives

1. Evaluate a PCR-based multiplex assay (Acuitas® CR Elite Test) of perianal swabs for detecting *bla* genes that confer carbapenem resistance
2. Compare PCR-based methods to culture based methods
3. Determine the feasability for a larger study comparing culture-based screening to a commercial real-time PCR-based strategy

Methods

The Acuitas® CR Elite Test (OpGen, Gaithersburg, MD) is a test that combines culture, identification, and antibiotic susceptibility testing (ID/AST) with the PCR-based multiplex assay gene test results of the Acuitas® MDRO Gene Test. The gene test results of CR Elite were used to detect *bla* KPC, NDM, VIM, IMP, OXA, and CTX-M gene families.



Perianal swab (Eswab, Copan Diagnostics, Brescia, Italy) samples were obtained from 12 patients admitted to the Detroit Medical Center who at time of enrollment did not have positive clinical cultures for CREs. Each sample was spiked with a single positive control at one of three concentrations. (See table). Bacterial isolates were clinical isolates from different geographical locations.

Organism	Resistance Determinant	Dilutions tested
K. pneumoniae	KPC-2	1.5x10^3, 1.5x10^5, 1.5x10^6
K. pneumoniae	OXA-48	1.5x10^3, 1.5x10^5, 1.5x10^6
P. aeruginosa	VIM	1.5x10^3, 1.5x10^5, 1.5x10^6
E. coli	NDM-1	1.5x10^3, 1.5x10^5, 1.5x10^6

Acuitas® MDRO Gene Test Analytical Sensitivity	
MDRO Genes	LOD (CFU/mL)
KPC	84
NDM	93
VIM	37-154
IMP	13-66
OXA-48	79
OXA-23	109
OXA-51	125
CTX-M	79-151
VanA	250

All specimens were “blinded” to microbiologic testers and tested in parallel using the Acuitas CR Elite Test, the CDC protocol, chromID CARBA agar, and SUPERCARBA agar. Cultures were interpreted as positive or negative if the colonies growing were either the predominant colony or if the individual colonies met the pre-defined criteria according to the manufacturer, the CDC, or the inventors for the chromID Carba, CDC Method, and SUPERCARBA media respectively.

Sensitivity to detect known controls and percent total concordance with the culture-based strategies were calculated.

Results

The genetic elements contained in the spiked isolates were identified by the Acuitas CR Elite Test in 12/12 (100%) cases containing KPC-2, OXA-48, NDM-1 and VIM. (Minimum bacterial load 1.5x10^3 organisms).

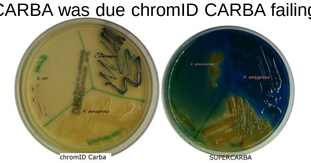
The CDC protocol identified carbapenem resistance in 7/12 isolates (58%). Discordance between the Acuitas CR Elite Test and the CDC protocol was due to susceptible determinations by the CDC protocol for isolates containing OXA-48, VIM, and NDM-1.

Results

The SUPERCARBA media identified carbapenem resistance in 9/12 isolates (75%). Discordance between Acuitas CR Elite Test and SUPERCARBA was due to SUPERCARBA failing to detect NDM in two specimens (low and high concentration), and failure to recover a VIM-producing *Pseudomonas* after an initial positive screen.

The chromID CARBA media identified carbapenem resistance in 8/12 isolates (66.7%). Discordance between Acuitas CR Elite Test and chromID CARBA was due chromID CARBA failing to recover all three NDM producers.

	Acuitas	chromID CARBA	SUPERCARBA	CDC Protocol
Sensitivity	100%	66.70%	75%	58%



The Acuitas test identified additional OXA elements in a spiked sample from a patient; however pre-existing colonization of the patient with an OXA-containing organism could not be ruled out. This isolate was not detected with any of the screening media.

Specificity was not calculated as a single negative specimen was included, which was properly identified by all methods.

Conclusions

The Acuitas® CR Elite Test was found to be a more sensitive method for the identification of pathogens containing clinically important carbapenemases.

Identification of *Pseudomonas* carrying *bla*VIM was particularly difficult with specialized media as published protocols call for the exclusion of non-lactose fermenters.

A rapid, sensitive, and accurate method for screening patients for colonization with MDR GNB could identity patients who are colonized and improve the capability of infection control programs to prevent the spread of MDR GNB pathogens.

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