Presentation: 284

Dr. C. Gordon
clg2171@columbia.edu

Development and validation of a multiplex PCR assay for rapid diagnosis of ventriculostomy-related infections

C.L. Gordon, MD[1,4], R. Tokarz, PhD[2], T. Briese, PhD[2], S. Whittier, PhD[3], J. Shah[1], M.T. Yin, MD[1]

[1]Division of Infectious Disease, Department of Medicine, [2]Center for Infection and Immunity, Mailman School of Public Health, Columbia University Medical Center; [3]Clinical Microbiology Service, New York Presbyterian Hospital; [4]University of Melbourne, Australia

Background

- Ventriculostomy related infection (VRIs) frequently occur causing significant morbidity and mortality.[1]
- Diagnosis of ventriculomeningitis is difficult because the clinical and CSF parameters are often also seen after intraventricular hemorrhage and neurosurgery[2], while there is the potential of serious neurological sequelae associated with delayed treatment.
- Most clinicians treat empirically with broad-spectrum antibiotics when there is clinical suspicion of a VRI.[3] These strategies promote the selection of resistant microorganisms[4], and expose the patient to the risks of potentially unnecessary antibiotics.
- We developed and validated a multiplex MassTag PCR assay for the rapid diagnosis of VRIs utilizing both broad-range bacterial and fungal primers and organism-specific primers. The process requires no special laboratory and is low cost.

Methods

- A retrospective review of culture-positive ventricular device (VD) CSF specimens at NYPH between 2001-2006 was performed to determine the most common pathogens.
- A 13-plex MassTag panel was developed targeting common VRI pathogens (Table 1). PCR primers were designed as previously described[5] and optimized using targeted sequences cloned into pCR2.1-TOPO or samples spiked with a known concentration of live organism. The level of detection ranged from 10^2 to 10^4 organisms per mL using samples spiked with known concentrations.
- The MassTag panel was then evaluated on serially collected clinical CSF specimens. 250uL of CSF was aliquoted into 750uL of lysis buffer under sterile conditions and stored at -70°C.
- Retrospective laboratory and chart review was performed to determine CSF and clinical parameters.
- Using accepted definitions,[1,6,7] VD patients were grouped into (1) definite VRI, (2) possible VRI, or (3) no VRI; while non-VD patients were grouped into (1) definite CNS infection, (2) possible CNS infection, or (3) no CNS infection.
- Contamination or colonization was defined as organism detection in a patient with no VRI or no CNS infection. Initial CSF samples were analyzed.

Table 1. VRI pathogens and gene targets

Organism	Gene Target
Staphylococcus (genus level)	16S rRNA
Staphylococcus aureus	femA
Methicillin-resistant *S. aureus*	mecA
Streptococcus pyogenes	speB
Propionibacterium acnes	recA
Enterococcus species	16SrRNA
Pseudomonas aeruginosa	ExoS
Enterobacter species	ompA
Acinetobacter baumannii	OXA-51
Serratia marcescens	ompA
Klebsiella species	ompA
Candida albicans	CaAGM1
Cryptococcus neoformans	Cap59

Table 2. Evaluation of VRI MassTag Panel

	Ventricular device present (n=49)		
Infection category	*No. samples tested*	*Positive MassTag*	*Organisms detected (No.)*
Definite VRI	9	6* (67%)	Coagulase-negative *Staphylococci* [CNS] (2), Methicillin-sensitive *S. aureus* [MSSA] (2), Methicillin-resistant *S. aureus* [MRSA] (1), *C. albicans* (1)
Possible VRI	18	6 (33%)	MSSA (1), MRSA (2), *Enterococcus* [ENT] (1), ENT & MSSA (1), MRSA or CNS (1)**
No VRI	22	7 (32%)	MSSA (1), ENT (4), *Klebsiella* [KLEB] (2)
	No ventricular device present (n=138)		
Definite CNS infection	6	3^ (50%)	MSSA (1), *Enterobacter* (1), *Cryptococcus* (1)
Possible CNS infection	9	0 (0%)	-
No CNS infection	123	12 (9.8%)	CNS (4), CNS or MRSA (1), MSSA (2) ENT (3), KLEB (1), ENT & CNS (1)

* Primers for the additional 3 organisms were not present on the MassTag panel. These included *Morganella morganii*, *Streptococcus parasanguinis* and *Micrococcus* species.

^ Primers for the additional 3 organisms were not present on the MassTag panel. These included *Streptococcus pneumoniae*, Group B *Streptococcus* and *Salmonella* species.

** MecA gene target does not differentiate between methicilin-resistant CNS or MRSA.

Results

- CSF from 186 subjects were analyzed: 49 with a VD (median age 43 years; interquartile range [IQR] 30-62 years; males 49%), and 137 without a VD (median age 39 years; IQR 10-59 years; males 47%).
- Among subjects with a definite VRI or CNS infection, MassTag detected a pathogen in 6/9 (67%) of cases, but in 6/6 (100%) of casesfor which there wa a primer matching the pathogen on the MassTag panel (Table 2).
- Among subjects with a possible VRI, the MassTag panel detected ≥1 pathogen in 33%.
- Among subjects with no VRI, an organism was detected in 32%, representing either contamination or VD colonization.
- Among subjects with no VD and no CNS infection, an organism was detected in 9.8%, mostly gram-positive organisms (Table 2). For *Staphylococcus* species, MRSA and MSSA, positive samples with highest signal, and thus highest pathogen load, occurred in all but one definite VRI or definite CNS infection groups, whereas considerably lower signal was observed in groups without infections. For *Enterococcus* species or *Klebsiella* species, detections with high signal values occurred in the VD group, suggesting colonization.
- The sensitivity for detecting a pathogen (that was part of the MassTag panel) in definite infection groups was 100% for both the VD group (6/6) and non-VD group (3/3). The specificity for the VD group was 76% (22/29) and 90% for the non-VD group.

Conclusions

The VRI MassTag panel performed well in definite VRI and CNS infection if the pathogen was on the panel. In addition, pathogens were detected with low signal strength in a third of cases without VRI, relating most likely to VD colonization. Further testing with more samples is required to evaluate detection of gram-negative and fungal pathogens.

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Funding: Irving Institute/CTO pilot award (UL1 TR000040), National Institutes of Health award AI057158 and the US Department of Defense.

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