Poster # 117

Culture-based Viability PCR: Strategies to Harness its Sensitivity and Minimize False Positives

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Abstract

Introduction: qPCR is a highly sensitive method for identifying the presence of potential pathogens. However, its utility as a tool to detect environmental contamination is limited by its inability to differentiate between viable and non-viable target cells.

Methods: We completed a prospective microbiological analysis of patient bed footboard samples at a tertiary care center using foam sponges and the stomacher method to process samples. Target species included E. coli (EC), S. aureus (SA), and C. difficile (CD). Sponge homogenates were split into three paths: 1) T0: 500uL was added to 4.5ml of species-specific (SS) broth; 500uL of the resulting mixture underwent DNA extraction and qPCR with SS primers, 2) T1: 500uL was added to 4.5mL of SS broth, and 3) Growth negative control (GNC): 500uL was added to 4.5mL of 8.25% sodium hypochlorite, incubated for 10 minutes, centrifuged for 15 minutes at 3100 RPM, then decanted and added to 5mL of SS broth after 2 PBS washes. T1 and GNC samples were then incubated at SS conditions (24 hours at 37C for EC and SA, and 48 hours for CD). After incubation, 500uL from T1 and GNC samples underwent DNA extraction and qPCR. All samples were also cultured on SS agar. A sample was considered viable for each species if 1) it was detected at T0, and the CT decreased by at least 1.0 at T1 compared to GNC or 2) it was undetected at T0, detected at T1, and undetected for GNC, or 3) grew on standard culture agar.

Results: 468 samples from 26 patient rooms were analyzed, including 156 for each species. Of the 26 original samples, 24 (92%), 11 (42%), and 2 (8%), had detectable levels of EC, SA, and CD via qPCR at T0 or T1, respectively, and could be assessed for viability. Of those, 3 (13%), 8 (73%), and 0 (0%) contained viable cells of EC, SA and, CD via qPCR, respectively. Notably, 5 (19%) of SA samples were culturable at T1, indicating broth enrichment enhances culture sensitivity; however, all were determined viable via qPCR as well.

Discussion: Culture-based viability PCR outperformed traditional culture methods in detecting viable pathogens with improved specificity compared to qPCR, highlighting its potential as a tool for assessing environmental contamination. Further large-scale studies are needed to confirm these results across different species.

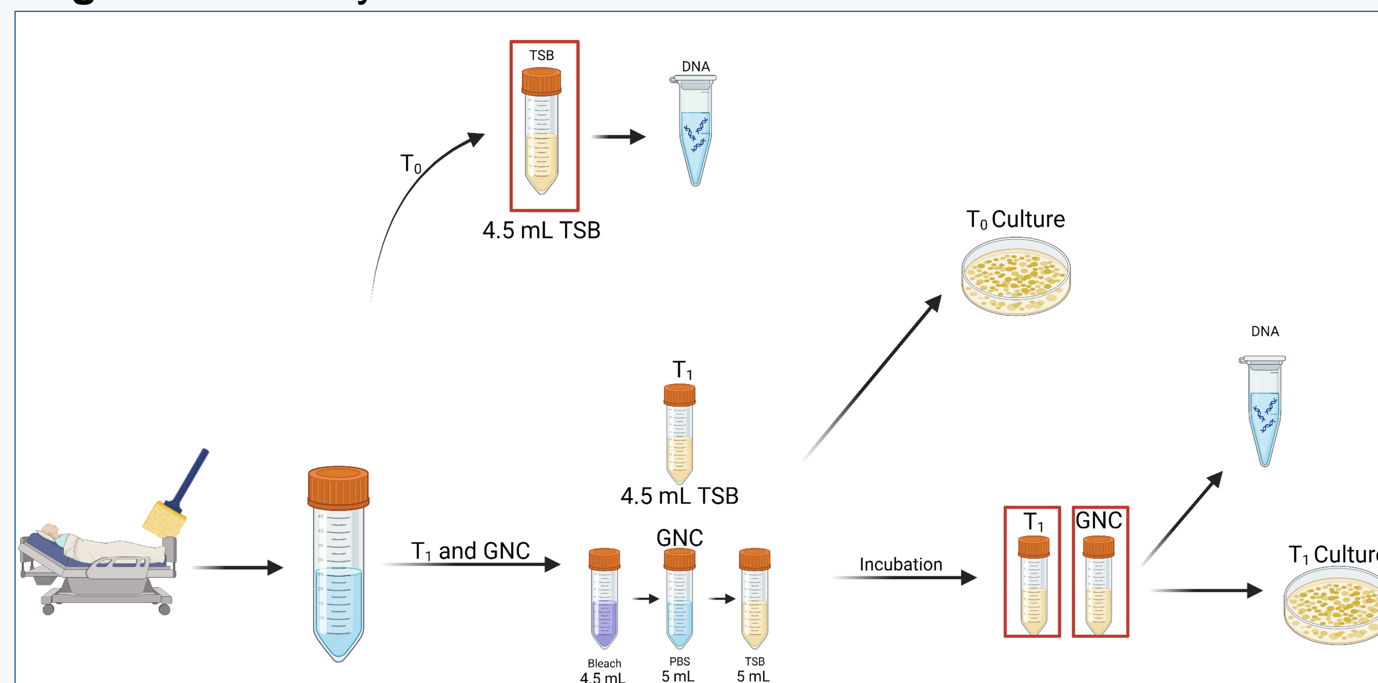
Background

- qPCR is a highly sensitive method for identifying the presence of pathogens
- However, its utility to detect environmental contamination is limited by its inability to differentiate between viable and non-viable target cells

Methods

- Prospective microbiological analysis of patient bed footboard samples at a tertiary care center using foam sponges and the stomacher method
- Target species: *E. coli* (EC), *S. aureus* (SA), and *C. difficile* (CD).
- Sponge homogenates were split into three paths:
  1. T_0: 500uL was added to 4.5ml of species-specific (SS) broth; 500uL of the resulting mixture underwent DNA extraction and qPCR with SS primers
  2. T_1: 500uL was added to 4.5mL of SS broth
  3. Growth negative control (GNC): 500uL was added to 4.5mL of 8.25% sodium hypochlorite, incubated, centrifuged, then decanted and added to 5mL of SS broth after 2 PBS washes
- T_1 and GNC samples were then incubated at SS conditions
- After incubation, 500uL from T_1 and GNC samples underwent DNA extraction and qPCR
- All samples were also cultured on SS agar
- A sample was considered viable if:
  1. Detected at T_0, and the CT decreased by at least 1.0 at T_1 compared to GNC
  2. Undetected at T_0, detected at T_1, and undetected for GNC
  3. Grew on standard culture agar

Figure 1. Viability PCR Methods



Results

- 468 samples from 26 patient rooms were analyzed, including 156 for each species.
- Of the 26 original samples, 24 (92%), 11 (42%), and 2 (8%), had detectable levels of EC, SA, and CD via qPCR at T0 or T1, respectively, and could be assessed for viability.
  - Of those, 3 (13%), 8 (73%), and 0 (0%) contained viable cells of EC, SA and, CD via qPCR, respectively.
- Notably, 5 (19%) of SA samples were culturable at T1 while not culturable at T0, indicating broth enrichment enhances culture sensitivity; however, all were determined viable via qPCR as well.

Table 1. Detection proportions and viability results by species, detection method, and study time point

	T_0		T_1		T_1 GNC		Viability
	Detected via qPCR n (%)	Detected via culture n (%)	Detected via qPCR n (%)	Detected via culture n (%)	Detected via qPCR n (%)	Detected via culture n (%)	Viable n (%)
Overall (N=78)	33 (42)	0 (0)	37 (47)	5 (6)	33 (42)	0 (0)	11 (14)
E. coli (N=26)	24 (92)	0 (0)	24 (92)	0 (0)	24 (92)	0 (0)	3 (11)
S. aureus (N=26)	7 (27)	0 (0)	11 (85)	5 (19)	7 (27)	0 (0)	8 (31)
C. difficile (N=26)	2 (8)	0 (0)	2 (8)	0 (0)	2 (8)	0 (0)	0 (0)

Conclusions

- Culture-based viability PCR outperformed traditional culture methods in detecting viable pathogens with improved specificity compared to qPCR, highlighting its potential as a tool for assessing environmental contamination.
- Further large-scale studies are needed to confirm these results across different species.