A Retrospective Evaluation of Conventional Culture versus Polymerase Chain Reaction in the Detection of Bacterial Species in Complex Lower Extremity Wounds

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PURPOSE

- The primary aim of this retrospective study is to compare the ability of conventional culture methods and polymerase chain reaction (PCR) to identify and quantify bacteria that contribute to lower extremity wound infections. We also sought to compare the difference in how culture and PCR reflect change of bacterial load with serial surgical debridement.

LITERATURE REVIEW

- Conventional culture methods pioneered by Louis Pasteur is the most commonly used method to identify bacteria for clinical decision making.
- Gene amplification of the 16S ribosomal RNA (rRNA) subunit by PCR and genetic sequencing has provided an innovative way of identifying and quantifying bacteria.
- Advantages of PCR include its sensitivity, rapidity, ability to detect organisms despite antibiotic therapy (1). The relative abundance of aerobic bacteria detected by PCR is reflective of the likelihood to be detected by culture in chronic wounds (2).
- Infections of the lower extremity present a unique challenge as they are often polymicrobial. To our knowledge, no study has undertaken a comparative evaluation of culture and PCR in the setting of complex lower extremity wounds that require serial debridement.

STUDY METHODS

- Deep tissue specimens were collected from 34 inpatients (**Table 1**) with complex lower extremity wound infections that required more than 1 operative debridement and IV antibiotics. Specimen were collected prior to (pre-) and following (post-) surgical debridement.
- Samples for culture were grown on conventional agar plates and phenotypic bacterial identification and estimated growth were provided.
- For the molecular diagnostics subset, the 16S portion of rRNA was amplified using PCR and pyrosequenced using Roche's FLX Titanium technology. Bacterial identification and quantification were provided to the tenth power.
- Senior author B.L. identified clinically relevant species from the obtained data. Inconclusive results from analysis were excluded. A growth code was assigned to evaluate quantitative trend with serial debridement (**Table 5**).
- McNemar's test was used to measure concordance of identification between culture and PCR. Kaplan-Meier survival curve was applied to characterize bacterial growth patterns with serial debridement.

RESULTS

- 124 matched samples were observed.
- 12 different species of bacteria were grown by culture, while PCR identified 15 species (**Table 2**).
- *Coagulase-negative Staphylococcus* (CoNS), *S. aureus*, and *Enterococcus spp.* were the most commonly identified by both methods **(Table 2).** The same results were observed when considering only those patients with diabetes (n=27).
- Culture identified *Enterococcus spp.* significantly more than PCR in data collected from OR1 (p=0.0082) **(Table 4)**. There was no significant difference in identification of CoNS, *S. aureus*, *S. agalactiae*, *and P. aeruginosa* between culture and PCR following OR1. No significant difference was observed in subsequent OR debridements.

Characteristics of study subjects	
Male	18 (52.9%)
Female	16 (47.1%)
Age	58.6±12.7
Mean Body Mass Index	33.2±8.8
Hb A1c	8.3±2.6
Diabetes Mellitus	27 (79.4%)
Hypertension	29 (85.29%)
Renal Disease	11 (32.35%)
Peripheral Arterial Disease	15 (44.12%)
# of Debridements (OR)	
1	3 (8.82%)
2	16 (47.06%)
3	12 (35.29%)
4	2 (5.88%)
5	1 (2.94%)
Mean number of ORs	2.5±0.7
# of matched samples (n)	124

Table 1: Study subject demographics

BACTERIAL DIVERSITY

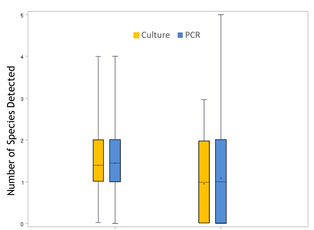

Figure 1: Difference in mean number of species detected from the initial OR to the final OR prior to closure. The mean number of species identified by culture significantly decreased from 1.40 to 1.0 (p=0.0188) and by PCR non-significantly decreased from 1.44 to 1.1 (p=0.1848).

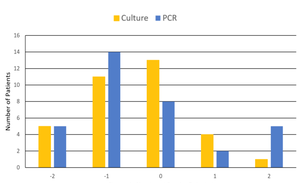

Figure 2: Frequency of difference in number of species detected from initial to final OR.

BACTERIAL IDENTIFICATION

Culture	
Bacteria result	**Frequency**
Coagulase-negative Staphylococcus	51
Staphylococcus aureus	38
Enterococcus sp.	38
Pseudomonas aeruginosa	16
Diptheroids sp.	12
Streptococcus agalactiae	9
Proteus mirabilis	8
Klebsiella Pneumoniae	7
Escherichia coli	6
Bacteroides fragilis	5
Proteus vulgaris	3
Enterobacter cloacae	1
Total	**194**

PCR	
Bacteria result	**Frequency**
Coagulase-negative Staphylococcus	44
Staphylococcus aureus	29
Enterococcus spp.	22
Corynebacterium stratum	19
Pseudomonas aeruginosa	17
Streptococcus agalactiae	16
Escherichia coli	16
Finegoldia magna	16
Proteus mirabilis	13
Bacteroides fragilis	11
Proteus vulgaris	4
Klebsiella Pneumoniae	2
Streptococcus viridans	1
Corynebacterium tuberculostearicum	1
Alcaligenes faecalis	1
Total	**212**

Table 2: Bacterial species identified by conventional culture and PCR. The same 3 most common bacterial species were identified by the two methods.

Wound location	N	Detected Bacteria	
		Culture (n)	PCR (n)
Hallux	2	*Proteus mirabilis (5)* *Strep. Agalactiae (4)* *Enterobacter cloacae (1)*	*Strep. Agalactiae (5)* *Proteus mirabilis (5)* *Enterobacter cloacae (1)*
Heel	6	*Coag neg Staph (7)* *Proteus vulgaris (3)* *Enterococcus spp. (14)* *Diphtheroids spp. (4)* *Proteus vulgaris (3)*	*Coag neg Staph (8)* *Proteus vulgaris (4)* *Enterococcus spp. (4)* *Proteus mirabilis (4)* *Escherichia coli (4)*
Plantar Foot	15	*Coag neg Staph. (30)* *Staph. Aureus (23)* *Enterococcus spp. (21)* *Diphtheroids spp. (8)* *Escherichia coli (6)*	*Coag neg Staph. (31)* *Staph. Aureus (19)* *Enterococcus spp. (17)* *Corynebacterium striatum (16)* *Finegoldia magna (12)*
Achilles	4	*Coag neg Staph. (6)* *Pseudomonas aeruginosa (6)* *Enterococcus spp. (2)*	*Pseudomonas aeruginosa (5)* *Coag neg Staph. 3* *Staph. Aureus (3)* *Enterococcus spp. (2)* *Bacteroides fragilis (1)*
Lower Leg	3	*Klebsiella pneumoniae (3)* *Staph. Aereus (3)* *Pseudomonas aeruginosa (2)* *Bacteroides fragilis (1)*	*Coag neg Staph (4)* *Escherichia coli (2)* *Klebsiella pneumoniae (1)*
BKA Stump	2	*Staph. aureus (5)* *Pseudomonas aeruginosa (4)* *Coag neg Staph (2)* *Enterococcus spp. (1)*	*Pseudomonas aeruginosa (5)* *Staph. aureus (3)*

Table 3: Bacterial species identified based on wound location.

Enterococcus			
	PCR		
Culture	0	1	Total
0	23 (67.7%)	0 (0%)	23 (67.7%)
1	7 (20.6%)	4 (11.7%)	11 (32.3%)
Total	30 (88.3%)	4 (11.7%)	34 (100%)
		McNemar's Test	
		P value	0.0082

Table 4: Enterococcus spp. was identified significantly more by culture than PCR in OR1.

QUANTITATIVE CHANGE

Culture Bacterial Growth	Growth Code	PCR Colony Forming Units (CFU)	CFU Code
No Growth	0	<10^5	1
Broth	1		
Scant	2	10^5-10^7	2
Few	3		
Moderate	4	>10^7	3
Heavy	5		

Table 5: A score is assigned based on detected abundance of bacteria by culture and PCR to quantify growth during each operative debridement.

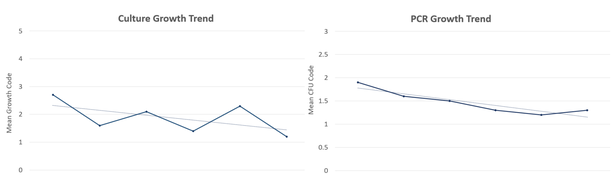

Figure 3: Comparison of average growth code detected by culture and PCR in complex lower extremity wounds that received IV antibiotics over the course of serial debridement. Significant decrease in growth trend was observed with culture (p=<.0001) and PCR (p=.0128) from initial to final OR closure.

DISCUSSION

- The index of organisms detected by PCR as compared to culture of complex surgical lower extremity wounds is sparse. Our data reflected no significant difference between culture and PCR in their ability to detect clinically relevant organisms in complex lower extremity wound infections undergoing treatment with IV antibiotics and serial debridement.
- Culture was more likely to detect *Enterococcus spp* than PCR in OR1. Largely regarded as a colonizer to have low virulence, a study has shown no difference in outcome when *Enterococcus spp* is not targeted with antibiotics (4). This may reflect the propensity of culture to cultivate clinically non-pathogenic bacteria during the first OR debridement, when wound contamination is conceivably highest. However, enterococcus may act as an opportunistic pathogen in wounds treated with antibiotics, which is reflected by its prevalence in both study groups.
- Culture revealed significant decrease in average number of species detected from initial OR visit to final debridement and wound coverage more so than PCR. This may pose clinical relevance with regard to targeted antibiotic selection.
- Significant reduction of bacterial load was displayed by both culture and PCR with serial debridement. The reported over-sensitivity of PCR may be related to detection of biodiversity, rather than quantitative assessment of bacterial load.
- Major differences in bacterial diversity were not observed. Rather, the two approaches may be complementary in their utility. Our data and review of the literature suggests that PCR may be useful in rapid identification of bacterial species during the first OR debridement, while culture may aid with evaluation of wound biodiversity with serial debridement. Future studies should delve into how a combined approach of PCR and culture, in conjunction with clinical factors, may be useful in clinical and operative decision making with complex lower extremity wounds.

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