Evaluation of 16S rDNA real-time PCR on seventy-one clinical samples in tertiary university hospital

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Background

Detection and identification of bacteria is nowadays based on **culture** and MALDI-TOF MS. However, **false negative** results may be obtained when bacteria in samples are *in vitro* compromised for growth (e.g. biofilms) or present in small amounts. Non-documented invasive infections implicate a delay in adequate therapy which may lead to higher morbidity and mortality rates. An alternative approach is the **molecular detection** of bacterial DNA by targeting the 16S ribosomal RNA gene (rDNA), followed by **sequencing** for identification. Here, we present an evaluation of a real-time PCR approach on 71 clinical specimens (41 patients).

Materials and methods

Most specimens were prosthetic joint (PJ) material, e.g. tissue, sonication and centrifugation fluids (n=54, 26 patients), 1 cerebrospinal fluid, 11 valve tissues (9 patients) and 5 samplings from other sites (5 patients).
Real-time PCR using TATAA SYBR® GrandMaster®Mix[1] enabled us to semi-quantitatively estimate the bacterial load based on **quantification cycle (C_q) values**. Sequencing was performed by GATC Biotech (Constance, Germany). Addition of a dsDNAse[2] treatment reduced bacterial genome contamination in reagents to a non-significant level. For all samples, DNA was extracted manually (QIAamp DNA Blood Mini[3]) after extensive cellular lysis (mutanolysine[4], proteinase K[4] or protease[3]) and overnight incubation.

For culture, specimens were inoculated within 4 h and incubated for 7-14 days in aerobic (5% CO_2) or anaerobic (Whitley Jar Gassing System) atmosphere at 35° C. **Colony-forming units (CFU) counts** were determined semi-quantitatively using the quadrant streak method.

Results

A Cq value lower than 43.8 (cutoff based on no-template controls) as well as a sequenceable amplicon were needed to consider a positive molecular result. We observed **32 culture-positive PJ** specimens **not confirmed** by **16S rDNA PCR**, mainly low amounts of coagulase-negative *Staphylococci* (coNS) or *P. acnes (table 1)*.
On the other hand, 16S rDNA real-time PCR yielded for **7%** of the samples a bacterial identification that had been **missed** by conventional **culture**. The clinical relevance of these was supported by identifications of the same species either in hemocultures or former samples. Interestingly, all patients received antibiotics.
Overall, the results of both methods agreed in about 50% of the specimens, with **26% congruent negative** results and **17% congruent positive** results with a **100% identity match** (MALDI-TOF MS vs. 16S rDNA sequencing). **Mean C_q** of the molecular positive samples was 24.0, which **differed significantly** (from non-identifiable samples (mean Ct 29.8, either due to no sequence obtained or a mixture).

Table 1. Bacterial identification of 71 clinical specimens with culture compared to 16S rDNA real-time PCR and sequencing.

Specimens*	Identification	Number of specimens (%)			
		Culture +			PCR +
		>20 CFU	≤20 CFU	Total	
Prosthetic joint specimens (n=54)	*S. aureus*	11	0	**11**	**10**
	coNS†	7	10	**17**	**1**
	E. coli	0	2¶	**2**	**0**
	E. faecalis	0	1¶	**1**	**0**
	P. acnes	6	7	**13**	**1**
	S. agalactiae	0	0	**0**	**1**
Valve tissues (n=11)	*E. faecalis*	1	0	1	1
	S. agalactiae	0	0	**0**	**1**
	S. gordonii	0	0	**0**	**1**
	C. burnetii	0	0	**0**	**1**
Other (n=5)	*P. micra*	0	0	**0**	**1**
total (n=71)		45 (63%)			17 (24%)

*: specimens with mutual negative culture and molecular analysis are not shown; †: S. epidermidis (n=14), S. capitis (n=1), S. pasteuri (n=2); ¶: only 1 CFU was cultured.

Conclusion

The highest **added** clinical **value** of 16S rDNA real-time PCR to culture is found for **samples** taken under **antibiotic treatment**. Although overall culture is more sensitive, culture-positive specimens, with low amounts of (low-virulence) bacteria, which are not confirmed by 16S rDNA PCR, may be a result of contamination. We suggest to perform 16S rDNA real-time PCR in these elective cases and always **in addition to bacterial culture**, e.g. when sampling after initiation of antibiotic therapy or when culture remains negative despite a high level of clinical suspicion for infection.

1. TATAA Biocenter (Gothenburg, Sweden); 2. ArcticZymes AS (Tromsø, Norway); 3. Qiagen (Hilden, Germany); 4. Sigma-Aldrich (St.Louis, Missouri, USA)

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