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Clinical utility of pan-microbial PCR assays in the routine diagnosis of infectious diseases

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ABSTRACT

The goals of the study were to examine the analytical properties and the clinical utility of pan-microbial PCR (PM-PCR) assays in a retrospective study conducted in 2014–2015 at the Tel-Aviv Sourasky Medical Center. PM-PCR included in-house assays for pan-bacterial, pan-fungal, and pan-mycobacterial PCR followed by sequencing. The clinical utility of the assays was decided based on defined criteria/categories. There were 585 PM-PCR tests performed on samples from 306 patients. The positivity rates of PM-PCR for bacterial, fungal, and mycobacterial infections were 72/316 (22.7%), 16/186 (8.6%), and 6/83 (7.2%), and the sensitivity values were 65%, 76%, and 85%, respectively. PCR results had influenced the management in 14/82 (17%) of PCR-positive cases and in 13/222 (5.8%) of PCR-negative cases ($P = 0.005$). The causes for the low clinical utility were related to lack of effect on the initial treatment in PCR-negative cases and concurrent positive cultures or presumed contamination in PCR-positive cases.

1. Introduction

Accurate detection and identification of microbial etiology are crucial parts in the management of infectious syndromes (e.g., pneumonia). In addition, since many clinical syndromes may be caused by infectious or noninfectious etiologies alike (e.g., arthritis), microbiological testing is sometimes required in order to "rule out" infectious etiology in order to allow the administration of other treatment modalities that otherwise may be detrimental in case of infection (e.g., immunosuppressive therapy). Although culture-based methods have been the mainstay of the microbiological diagnostic process, these methods have several important limitations. The sensitivity of cultures might be low for several reasons: 1) uncultivable/difficult-to-culture organisms (e.g., *Legionella* spp., *Pneumocystis jirovecii*), 2) low-inoculum infections, and 3) previous antimicrobial treatment. Another important drawback of cultures is the relative long turnaround time (TAT), which may be particularly problematic in slow-growing organisms (e.g., *Mycobacteria* spp.).

The application of nucleic acid amplification tests (NAATs) in clinical microbiology had provided solutions to many of these problems. NAATs are now commonly used in the diagnosis of fastidious organisms (Murdoch et al., 2013) and allow rapid diagnosis of infections caused by slow-growing organisms (Chakravorty et al., 2017). However, many clinical syndromes (e.g., hospital-acquired pneumonia) are potentially caused by a variety of different organisms; thus, pathogen identification in such cases requires the ability to detect a large variety of organisms.

One solution that allows the detection of a variety of organisms is a broad-range or "pan-microbial" (PM) PCR. Such assays are based on direct-sample amplification of genes that are both present in all members of a particular phylum and possess sufficient variability that allows differentiation of species within that phylum (Sontakke et al., 2009). Following an amplification stage using primers whose sequences are preserved throughout the phylum, the identity of the species is further determined by sequencing of the amplicon and comparison of the results with phylogenetic database (e.g., leBIBI (Devulder et al., 2003)). Commonly used targets are the 16S rRNA as pan-bacterial PCR (Rampini et al., 2011), the interspacer region as pan-fungal PCR (Rampini et al., 2016), and a subset of the 16S rRNA or HSP65 gene PCR that is designed for mycobacterial identification (Opota, 2017). Although these methods have been developed and studied over the last 2 decades (Sontakke et al., 2009), there are limited data regarding their exact role in the diagnostic scheme (e.g., concomitant with cultures or as an ancillary test for culture-negative samples (Rampini et al., 2011)) or their clinical utility (Akram et al., 2017).

Our center has been using PM-PCR assays (pan-bacterial, pan-fungal, and pan-mycobacterial PCR) for more than 5 years. The goals

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of this work were to study the analytical performances of these tests in comparison with cultures in a large-scale study and to analyze their clinical utility in relation with the demographic and clinical characteristics of the corresponding patients.

2. Materials and methods

2.1. Study design

This was a retrospective study designed to evaluate the analytical parameters of PM-PCR (pan-bacterial, pan-fungal, and pan-mycobacterial PCR) tests and their effect on patient's management. The study included all PM-PCR tests sent from samples of patients that were admitted to the Tel-Aviv Sourasky Medical Center (TASMC) in 2014–2015. TASMC is a 1400-bed tertiary-care center that serves the population of the Tel-Aviv area. PM-PCR tests are sent to an external service laboratory following approval of an Infectious Diseases specialist, although there are no clear indication (e.g., negative culture) for test ordering. The median TATs are typically 4 and 6 days for a negative and positive PM-PCR tests, respectively. The study was approved by the TASMC Ethical Committee.

2.2. Data collection and determination of test's effect on patient's management

PM-PCR tests were identified by the laboratory information system. For each patient identified, data were collected from the TASMC computerized systems including microbiological, demographic, and clinical data. The latter included antimicrobial treatment (prior and following sample collection), the presumptive infectious disease diagnosis for which the patient was tested, the effect of the PM-PCR results (i.e., treatment changes), and patients' outcome (based on the index admission and subsequent clinic/hospital admissions).

The effect of the PM-PCR results on patients' management was determined according to defined arguments (see Table 4) independently by 2 Clinical Microbiologists physicians. The initial review was followed by chart review, and deliberation of contested cases until resolution was obtained.

2.3. Microbiological and molecular methods

Bacterial (aerobic and anaerobic), fungal, and mycobacterial cultures were performed routinely at the TASMC laboratory, with the vast majority of cultures processed within several hours from collection. Samples for anaerobic cultures were not transported with Anaerobic Transport System, and anaerobic cultures were done using jars. Identification of bacterial and yeast isolates was done using VITEK 2 or the VITEK MS systems (bioMérieux, Marcy l'Etoile, France); molds were identified based on morphologic features, and *Mycobacteria* other than tuberculosis (MOTT) were identified at the Israeli Mycobacteria reference laboratory.

PM-PCRs were performed as send-out tests at the Rambam Medical Center. The tests were performed by in-house assays and are based on amplification of a broad-spectrum target (e.g., 16SrRNA) followed by sequencing of positive amplicons and comparison of the sequence with the NCBI database by the Basic Local Alignment Search Tool. Three different PM-PCR tests were performed: 1) pan-bacterial (Shachor-Meyouhas et al., 2013), 2) pan-fungal (Kadmon et al., 2012), and 3) pan-mycobacterial PCR (Kassis et al., 2007). The sensitivity and specificity of the pan-bacterial PCR were 46% and 98%, respectively, compared with blood cultures (Shachor-Meyouhas et al., 2013). The detection rate of clinically significant fungi (excluding *Pneumocystis jirovecii*) by the pan-fungal PCR in broncheoalveolar lavage was approximately double compared with cultures (Kadmon et al., 2012). The pan-mycobacterial PCR had been evaluated for identification but not for the direct detection of mycobacteria (Hall et al., 2003; Kassis et al., 2007). The laboratory TAT was 1–2 days for a negative test and 3–4 days for a positive test. Including shipment (average 2 days), the actual TAT was 3–4 days and 5–6 days for a negative and positive tests, respectively. The technical details of the tests are entailed in their corresponding publications. Only results that were obtained by PM-PCR (but not other species-specific PCR) were included in the study.

2.4. Data analyses

Since the majority of tests were ordered for culture-negative samples (see 3.2), the sensitivity values of the PM-PCR tests were calculated using the combined positive results of cultures and PM-PCR as the denominator (i.e., true positive). As the number of mismatched positive results (i.e., culture vs. PM-PCR) was relatively small and due to the difficulties in defining "false-positive" PM-PCR results, specificity was not calculated.

To identify the factors that could predict an effect of the PM-PCR tests on patients' management, a case–control study was performed. All variables were analyzed as categorical (including age) by χ^2 test using β level of ≤0.05. Similar analysis was done to study the correlations with the PM-PCR positivity.

3. Results

3.1. General features of pan-microbial PCR assays

During the study period (2014–2015), 694 PM-PCRs were sent for 351 patients, of which 45 patients (109 tests) were excluded from the study due to poor documentation. Hence, the study included 585 PM-PCRs sent for 306 patients, which constitute less than 1% of the corresponding cultures (e.g., respiratory, CSF cultures, etc.). The distribution of tests according to year, type, and results is presented in Fig. 1. The total number of positive tests was 94/585 tests (16%) and was significantly higher in pan-bacterial vs. the other PM-PCR assays ($P < 0.001$).

The distribution of tests according to source of sample, type of PM-PCR, and positivity rate is presented in Table 1. The most common source was the musculoskeletal system ($n = 184$), mostly from synovial fluid ($n = 94$), followed by the central nervous system ($n = 150$), mostly from the CSF ($n = 95$). The numbers of positive tests were significantly higher in samples from ear, nose, and throat (ENT) (12/27, 44.4%, $P < 0.001$) and the lower respiratory tract (LRT) (20/80, 25%, $P = 0.03$)

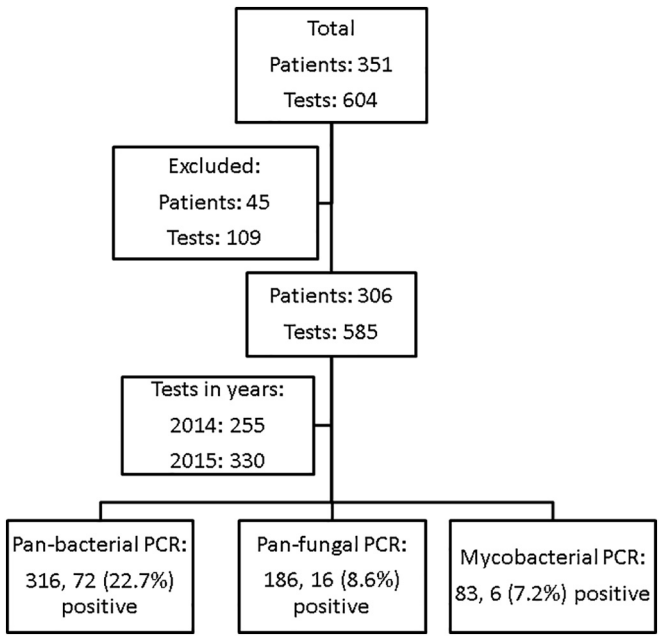

Fig. 1. Selection of pan-microbial PCR tests and their distribution according to year, type, and positivity rate.

Table 1
Distribution of tests according to source of sample, type of PM-PCR, and positivity rate.

Service/system	Total test number	Material (number)	Positive PCR tests (%)	PCR test type		
				Bacterial DNA PCR	Fungal DNA PCR	NTM DNA PCR
Musculoskeletal	184		15 (8.15%)	97	57	30
		Synovial fluid (94)	6			
		Bone (44)	2			
		Soft tissue (20)	4			
		SSI (21)	3			
		Foreign body (5)	0			
Neurology, neurosurgery	150		18 (12%)	90	47	13
		CSF (95)	7			
		White matter (33)	8			
		SSI (12)	1			
		Dura matter (6)	2			
		Cranium (2)	0			
		Foreign body (2)	0			
Lower respiratory tract	80		20 (25%)	37	28	15
		Bronchial lavage (46)	11			
		Lung tissue (16)	2			
		Pleural fluid (15)	4			
		Sputum (3)	3			
Dermatology	60		12 (20%)	32	21	7
Internal medicine/hematological	41		9 (21.95%)	22	11	8
		Lymph tissue (40)	9			
		Blood (1)	0			
ENT	27		12 (44.4%)	14	10	3
		Ear (9)	6			
		Nose (6)	2			
		Sinuses (5)	2			
		Thyroid gland tissue (4)	2			
		Neck (3)	0			
Gastroenterology, general surgery	25		4 (16%)	13	7	5
		Liver (14)	2			
		SSI (4)	0			
		Peritoneal fluid (3)	2			
		Breast tissue (2)	0			
		Spleen (2)	0			
Cardiology	10		2 (20%)	7	2	1
		Valves (6)	2			
		Pericardium (4)	0			
Urology	4		0 (0%)	2	1	1
		Kidney (3)	0			
		Fluid in cyst near urinary bladder (1)	0			
Ophthalmology	2		0 (0%)	1	1	0
OMFS	2		2 (100%)	1	1	0
Total, *n* (% positivity)	585 (16)			316 (29)	186 (9.4)	83 (7.8)

NTM = nontuberculous mycobacteria, CSF = cerebrospinal fluid, SSI = surgical site incision.

and significantly lower in samples from the musculoskeletal system (15/184, 8.1%, $P < 0.001$).

3.2. Microbiological features and concordance with cultures of pan-microbial PCR

The microorganisms detected by the PM-PCR are presented in Table S1. There were 15 reports of anaerobic bacteria (20% of all pan-bacterial results) and additional 7 reports of relatively fastidious organisms (e.g., *Haemophilus influenza*, *Nocardia* spp.). Pan-fungal PCR results were divided almost evenly between *Aspergillus* spp. and various yeasts; 3 (50%) of the mycobacteria detected were related to temperature-sensitive species, i.e., *M. marinum* and *M. haemophilum*.

Relevant cultures were performed concomitantly on all PCR-positive samples with the exception of 12 tests (3 bacterial and 9 fungal) and were positive in 39/94 (41.4%) of PCR-positive samples, but the organism was identical to the PCR results in only 27/94 of cultures (28.7%) (Table S1). The same organism was detected in cultures that were collected from other sites (e.g., blood and CSF) in additional 9 cases, adding altogether to 36/94 (38.2%) concordance of cultures and PM-PCR results. Culture concordance was especially common in pan-mycobacterial PCR, where 5/6 cases were also recovered by culture.

Since ENT- and LRT-related samples had the highest rates of positive PCR, we analyzed the organisms that were detected in these samples. Among ENT-related samples, 5/12 yielded relatively fastidious organisms (*Fusobacterium necrophorum*, *Streptococcus pneumoniae,* and *M. haemophilum*) that did not grow in cultures. Most of the other organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) either grew also from other cultures or were considered insignificant (*S . epidermidis*).

In the PCR-positive LRT samples ($n = 20$), notable organisms included 3 MOTT that were also recovered from cultures, 4 *Aspergillus* spp. that were not recovered from cultures, and 3 fastidious bacteria that were not recovered from cultures (*Nocardia, Actinomyces* spp., and *F. nucleatum*). *S. pneumoniae* was reported in 4 samples of which 3 were also recovered by cultures.

In 44/491 tests (8.9%), culture was positive despite negative PCR. Bacterial isolates included *S. aureus* ($n = 7$), other staphylococci ($n = 8$), viridans group streptococci and enterococci ($n = 4$), gram-negative bacilli (*P. aeruginosa*, *Escherichia coli*, and *Stenotrophomonas maltophilia*, $n = 4$), and various species that were regarded as contaminant (e.g., *Bacillus* spp.). There were 6 fungal isolates (*A. fumigatus*, *Candida albicans*, *Penicillium,* and *Trychophyton* spp.) and 1 mycobacterial isolate (*M. simiae*); additional mycobacterium (*M. marinum*) was isolated from a specimen that was tested (and reported negative) for

Table 2
Relation between demographics and antimicrobial treatment and test results.

		Total patient number	Total test number	Growth in culture (%)	Positive PCR result (per patient) (%)
Age	Under 18	106	172	23 (21.7%)	32 (30%)
	Above 18	200	413	59 (29.5%)	44 (22%)
Sex	Male	176	336	48 (27.3%)	41 (23.3%)
	Female	130	249	33 (25.4%)	35 (27%)
Antimicrobial treatment 3 d prior to specimen collection					
	Yes	225	481	63 (28%)	59 (26.2%)
	No	81	167	18 (22%)	17 (21%)

pan-bacterial DNA only. Based on the combined culture and PCR results, sensitivity was calculated as follows: pan-bacterial PCR, 65%; pan-fungal, 76%; and pan-mycobacterial PCR, 85%. Notably, in musculoskeletal samples, there were 5 pan-bacterial PCR-positive/culture-positive samples, 8 pan-bacterial PCR-positive/culture-negative samples, and 14 pan-bacterial PCR-negative/culture-positive samples, resulting in a pan-bacterial PCR sensitivity rate of only 15/29 (48%).

3.3. Clinical variables related to study subjects

The relations between demographics, antimicrobial treatment, and tests results are presented in Table 2. The study included both adult (65%) and pediatric patients (35%); while in the latter, the growth rate in concurrent culture was lower (21.7% vs. 29.5%), the rate of positive PCR tests was higher (30% vs. 22%, $P = 0.23$). The rates of positive PCR tests were similar in male and female patients. In the majority of cases (225/306, 73.5%), the samples were collected while patients were treated with antimicrobials, albeit this had no significant effect on the rate of positive cultures or PCR.

3.4. The effect of PCR results on patients' management

All cases were reviewed independently by 2 Clinical Microbiologists (MDs, A.A. and S.A.) and were categorized according to the effect of tests results on patients' management using predetermined criteria. The categorization differed in 50 cases, and following repeated data review and deliberation, 48 contested cases were resolved and 2 cases (with 6 tests) remained contested (Table 3). The PCR results had no influence on the management in the majority of cases (277/304, 91.2%); in PCR-positive cases, the results influenced the management in 14/82 (17%) cases (Table 4), and in PCR-negative cases, the results influenced the management in 13/222 cases (5.8%, $P = 0.005$). PM-PCR–positive tests that had an effect on clinical management ($n = 14$, Table 4) were characterized by the presence of mostly fastidious organisms and were overall related to severe infections (e.g., 4 cases of intracranial infections). Cultures were negative (or interpreted as contaminants) in all of these cases, with the exception of patient 85 were *M. kansasii* was also reported by culture, albeit later than PCR results were reported. The identification of a specific pathogen had allowed either to initiate a specific treatment or to narrow the spectrum of the antimicrobial regimen.

The most common causes for lack of effect of PCR-positive tests on patient's management were concurrent positive cultures or presumed contamination. In the latter, sequencing results yielded mainly commensal bacteria (e.g., coagulase-negative staphylococci and viridans group streptococci), various fungi (*Candida* and *Cryptococcus* other than *neoformans* spp.), but also potentially pathogenic bacteria (e.g., *S. aureus*, *Actinomyces* spp.) that were regarded as irrelevant to the patient's clinical condition. In the majority of PCR-negative cases (173/224, 77.2%), no change was made in the initial management, i.e., antimicrobials were continued in the vast majority of cases. Effect on management was more common in pan-bacterial PCR (22/314, 7%, $P = 0.037$) compared with pan-fungal (6/184, 3.2%) or pan-mycobacterial-PCR (2/81, 2.4%).

We performed a case–control study to examine the effects of sample source, demographics, and recent antimicrobial treatment on the clinical utility of the tests; none of these factors was found to be related to increased test utility (Table 5). Similarly, concurrent growth in culture was not associated with a change in the clinical utility of the tests (data not shown).

4. Discussion

Our study presents the first comprehensive, combined analysis of all 3 PM-PCR assays (pan-bacterial, pan-fungal, and pan-mycobacteria PCR) and the second largest study of PM-PCR in the literature (Rampini et al., 2011). Moreover, with the exception of 2 small studies (Akram et al., 2017; Alraddadi et al., 2013), this study is the first to provide a large-scale analysis of the clinical utility of these tests.

Our study analyzed samples from a variety of sources, thus allowing us to compare the relative source-related yield. The overall positivity rate was 16%, but specific rates varied significantly according to the type of PM-PCR (pan-bacterial, 22.7% >> pan-fungal and pan-mycobacterial PCR, ~8%) and the source of samples (ENT, 44.4% > LRT, 25% > musculoskeletal, 8.1%). The overall sensitivity of pan-bacterial PCR was 65%, but this value varied too according to source, being lower in musculoskeletal samples (48%) and higher in ENT samples (86%, data not shown), including 5 cases of otogenic infections by *Fusobacterium* spp., of which none grew in cultures. The concordance between PM-PCR and cultures was relatively low: There were relatively large numbers of fastidious and anaerobic bacteria found in the culture-negative/PCR-positive samples. Of the 39 culture-positive/PCR-positive samples, only 27/39 samples (69%) showed concordant results, suggesting a high rate of contamination of either cultures and/or PCR. Even more surprising (considering that PM-PCRs were ordered as ancillary special tests in less than 1% of cultures) was the high number of culture-positive/PCR-negative samples ($n = 44$).

Direct comparison with previous studies is problematic due to the vast differences in studies' designs and population, and therefore, we

Table 3
The effect of pan-microbial PCR tests on patients' management.

PCR result	Patients/tests, no.	Decision (per patient)		Argument	No.
Positive	82/94	The results had influence on patients' management	14	Positive tests, therapy was adjusted.	14
		The results didn't have influence on patients' management	68	Presumed contamination or not compatible with clinical picture.	27
				Positive test, positive culture.	25
				Positive test, positive culture of another specimen.	3
				Positive test, initial therapy not altered.	6
				Positive test, results received after discharge/death.	8
Negative	224/491	The results had influence on patients' management	13	Negative test, therapy altered or stopped	13
		The results didn't have influence on patients' management	209	Negative test, no change in initial management.	173
				Negative test, positive culture from different specimen.	18
				Negative test, positive culture.	13
				Negative test, diagnosis was based on another test.	5
		No agreement	2		

Table 4
Clinical features of PCR-positive cases where PCR had an effect on the management.

Case #	Age/gender	Diagnosis	Sample	Previous abx.	PCR I.D.	Effect of PCR on management and follow-up
22	15/M	Subdural abscesses	CSF	Yes	*Bacteroides* spp.	Vancomycin discontinued; pt. recovered.
23	51/F	Chronic osteomyelitis	Bone biopsy	Yes	*Enterococcus faecalis*	Treatment extended to 8 wk; pt. recovered.
24	15/M	Subdural abscesses	Pus, brain	Yes	*Haemophilus influenzae*	Vancomycin discontinued; pt. recovered.
39	1/M	Brain abscess	Pus, brain	Yes	*Candida albicans*	Meropenem discontinued, fluconazole initiated; pt. improved.
83	1/M	Mastoiditis, brain abscess	Pus, mastoid	Yes	*Streptococcus pneumoniae*	Metronidazole discontinued; pt. recovered.
85	21/F	Chronic pneumonia	Sputum	Yes	*Mycobacterium kansasii*	Specific antimicrobials initiated; outcome unknown (transferred to other institution).
99	40/M	Wound infection	Pus, wound	Yes	*Nocardia* spp.	Vancomycin discontinued, TMP-SFX initiated; pt. improved.
118	24/F	Lung lesion, febrile neutropenia	Lung biopsy	Yes	*Aspergillus* spp.	Piperacillin-tazobactam discontinued, voriconazole initiated; pt. improved.
130	2/F	Pneumonia	Pleural effusion	Yes	*Streptococcus pneumoniae*	Clindamycin discontinued, amoxicillin initiated; pt. recovered.
136	34/M	Septic arthritis	Swab, wound	No	*Streptococcus pyogenes*	Ciprofloxacin discontinued; no follow-up.
151	2/M	Pneumonia	Pleural effusion	No	*Streptococcus pneumoniae*	Clindamycin discontinued; pt. recovered.
155	62/F	Septic arthritis	Joint biopsy	No	*Gemella bergeri*	Ceftazidime discontinued; pt. recovered.
160	17/M	Lung lesion, febrile neutropenia	Bronchial lavage	Yes	*Aspergillus* spp.	Antibacterials discontinued; pt. improved.
231	37/M	Chronic pneumonia, HIV	Bronchial lavage	Yes	*Nocardia cyriacigeorgica*	Ceftriaxone discontinued, TMP-SFX initiated; pt. improved.

looked into studies that included a variety of samples and on studies that focused on 2 specific sources: musculoskeletal and ENT samples. In a large prospective study (Rampini et al., 2011), the overall sensitivity of pan-bacterial PCR was 84.5%, but the sensitivity from negative cultures was 42.9%. There were no discordant results in culture-positive/PCR-positive samples, and unlike in our study, there were only 3/18 anaerobic bacteria in the culture-negative/PCR-positive samples. In the study by Akram et al. (2017), the sensitivity of pan-bacterial PCR was 58%; the number of positive tests was too small to analyze concordance or species representation.

An extensive study of prosthetic joint samples found a positivity rate of pan-bacterial PCR of 123/497 (24%) and a sensitivity of 75.9%, while 2 studies that focused on wider range of musculoskeletal samples showed positivity rates of 7/36 (19%) (Alraddadi et al., 2013) and 36/104 (34%) with a sensitivity of 88.5% (Lagier et al., 2015). These results fare much better compared with our study where the pan-bacterial positivity rate and sensitivity were 13/97 (13.4%) and 48%, respectively. The extent of data regarding PM-PCR in ENT samples is much smaller. A small study of 9 patients found good concordance between pan-bacterial PCR and cultures but little added value of PCR over cultures (Westergren et al., 2003). An additional small study of 20 patients with chronic sinusitis reported positive pan-bacterial PCR in all 15 culture-negative/PCR-positive samples, but the predominance of *Staphylococcus* spp. raises the concern for high rate of contamination in that study (Karunasagar et al., 2018). Regarding otogenic infections, *Fusobacterium* spp. that was found in our study by PCR alone in 5 cases was commonly retrieved by cultures in other studies (Le Monnier et al., 2008; Yarden-Bilavsky et al., 2013), which suggest suboptimal conditions for anaerobic cultures in our laboratory.

The clinical utility of PM-PCR was analyzed in only 2 small studies. In the first study that focused on culture-negative bone and joint infections (Alraddadi et al., 2013), the positivity rate was 7/36 (19.4%), and the management was changed in 19 PCR-negative patients and in 7 PCR-positive patients (overall, 26/36, 72%). This rate was much higher than the rate found in our study in musculoskeletal samples (8/81, 9.8%), probably as a result of 1) higher positivity rate and 2) higher rate of antimicrobial discontinuation in PCR-negative patients. This is in stark contrast to our study where overall treatment was discontinued following negative PCR in only 13/224 (5.8%) patients, whereas in 209/224 (93%) cases, the treatment was not altered. Considering the low sensitivity and negative predictive value (NPV) of pan-bacterial PCR in culture-negative samples from clinically infected patients (42.9% and 80.2%, respectively) (Rampini et al., 2011), it is hard to see the justification for treatment discontinuation based on negative PCR results. In the

Table 5
Case–control study of the effect of pan-microbial PCR tests on patients' management.

Variables (*n*)		The results had influence on patients' management (%)	The results didn't have influence on patients' management (%)	*P* value
Service/system	Orthopedics (81)	8 (10)	73 (90)	0.8197
	Neurology/neurosurgery (85)	8 (9)	77 (91)	0.8247
	Pulmonology (42)	7 (16)	35 (84)	0.0750
	Dermatology (30)	2 (7)	28 (93)	1.0000
	Infectious diseases/internal medicine (23)	0	23 (100)	0.2425
	ENT (16)	2 (12)	14 (88)	0.6423
	Gastroenterology (13)	0	13 (100)	0.6147
	Cardiology (7)	0	7 (100)	1.0000
	General surgery (3)	0	3 (100)	1.0000
	Urology (2)	0	2 (100)	1.0000
	Ophthalmology (1)	0	1 (100)	1.0000
	OMFS (1)	0	1 (100)	1.0000
Antimicrobial treatment 3 d prior to specimen collection	Yes (224)	19 (8)	205 (92)	0.6530
	No (80)	8 (10)	72 (90)	
Age	Under 18 (198)	17 (8)	181 (92)	0.8340
	Above 18 (106)	10 (9)	96 (91)	
Sex	Male (174)	16 (9)	158 (91)	1.0000
	Female (130)	11 (8)	119 (92)	

other more recent study of both culture-negative and -positive samples (Akram et al., 2017), antimicrobial therapy was rationalized after positive PCR results in 5 patients (15.6%). However, since cultures were also positive in 3/5 samples, it is hard to argue for the added value of the PCR results in these cases. Antimicrobials were ceased in 4 cases (12.5%) based on negative PCR results, which again is hard to justify based even on their results of sensitivity and NPV of 58% and 77.7%, respectively. Therefore, based on our approach, a clear effect of PCR can be definitely argued in only 2/32 cases (6.2%), similar to our findings.

We identified additional major causes for low clinical utility of PM-PCR (Table 3), which included 1) PCR-positive results that most likely represented contamination/colonization (27/82, 32.9%) and 2) PCR-negative results where the diagnosis was made based on positive culture results or other microbiological tests (e.g., serology) (36/209, 17.2%). Together, they highlight the problems of using PM-PCR that stems from the presumed low clinical specificity and low analytical sensitivity, respectively.

Our study has several limitations. First, its retrospective design and the subjective manner at which the clinical utility was determined might have undermined the accuracy of the data and allowed subjective bias to influence the decisions. These problems were addressed first by basing our study on data that are mostly objective and relatively easy to collect (e.g., microbiological data, antimicrobial treatment) and by applying a process of dual, independent review of the data by two Medical Microbiologists. Second, PM-PCR application in clinical practice was not done according to defined criteria or algorithm, thus making it much harder to deduce conclusion regarding both its analytical properties and clinical utility. Therefore, when comparing our results to previous studies, we tried to "normalize" our measurements according to the design used in those studies. Third, since PM-PCRs were used as "send-out" tests, their TAT has relatively long, thus abating some of its potential advantages as "rapid tests." Fourth, the number of PM-PCR tests included in our study for some types of infections, e.g., valve tissue for endocarditis, was too low to allow comparison with previous studies (Kim et al., 2017).

Therefore, what can be an efficient way to implement PM-PCR in routine diagnostics? Considering the limitations of our study, we can only draw general outlines of "do's and don'ts". First, due to their relatively low sensitivity in culture-negative infections (Rampini et al., 2011), PM-PCR should not be used to "rule-out" infection, either altogether (i.e., to discontinue treatment) or partially (i.e., de-escalation of treatment). Second, since PM-PCR cannot provide antimicrobial susceptibility results, even positive identification of species might be of limited value in infections where the susceptibilities patterns of the relevant pathogens are less predictable, e.g., Enterobacteriaceae in intra-abdominal infections. Third, due to the innate limitations of PM-PCR in identifying polymicrobial infections, their value is lessen in infections that are typically polymicrobial, and therefore, even the identification of one species cannot allow narrowing the treatment for that species alone. For example, the identification of a *Streptococcus* species in a brain abscess sample would not (and should not) allow the discontinuation of antianaerobic treatment. Fourth, since the TAT of PM-PCR is not expected to be shorter compared with bacterial cultures, it is less likely to provide benefit in cases of infections that does not require prolonged treatment. Therefore, a useful application is most likely to be achieved in cases of culture-negative, clinically established significant infections, where antimicrobial treatment is expected to be prolonged, and the infection is likely to be monobacterial. Examples that are based on our study include empyema or other chronic lung infections (e.g., nocardiosis), otogenic and other focal intracranial infections, etc.

In addition to their diagnostic utility, the use of PM-PCR in our laboratory had allowed us to identify several important shortcomings in our culturing methodology, such as the isolation of strict anaerobes or *Nocardia* species. Although it is beyond the scope of this manuscript, the role of PM-PCR approach vs. other molecular diagnostic alternatives (e.g., "syndrome-based," species-specific multiplex PCR (Leber et al., 2016)) needs to be better defined.

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