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ORIGINAL ARTICLES

Polymerase Chain Reaction–Electrospray–Time-of-Flight Mass Spectrometry Versus Culture for Bacterial Detection in Septic Arthritis and Osteoarthritis

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Background: Preliminary studies have identified known bacterial pathogens in the knees of patients with osteoarthritis (OA) before arthroplasty. *Aims:* The current study was designed to determine the incidence and types of bacteria present in the synovial fluid of native knee joints from adult patients with diagnoses of septic arthritis and OA. *Patients and Methods:* Patients were enrolled between October 2010 and January 2013. Synovial fluid samples from the affected knee were collected and evaluated with both traditional microbial culture and polymerase chain reaction–electrospray ionization–time-of-flight mass spectrometry (molecular diagnostics [MDx]) to prospectively characterize the microbial content. Patients were grouped by diagnosis into one of two cohorts, those with clinical suspicion of septic arthritis ($n=44$) and those undergoing primary arthroplasty of the knee for OA ($n=21$). In all cases where discrepant culture and MDx results were obtained, we performed species-specific 16S rRNA fluorescence *in situ* hybridization (FISH) as a confirmatory test. *Results:* MDx testing identified bacteria in 50% of the suspected septic arthritis cases and 29% of the arthroplasty cases, whereas culture detected bacteria in only 16% of the former and 0% of the latter group. The overall difference in detection rates for culture and MDx was very highly significant, p-value $= 2.384 \times 10^{-7}$. All of the culture-positive cases were typed as *Staphylococcus aureus*. Two of the septic arthritis cases were polymicrobial as was one of the OA cases by MDx. FISH testing of the specimens with discordant results supported the MDx findings in 91% (19/21) of the cases, including one case where culture detected *S. aureus* and MDx detected *Streptococcus agalactiae*. *Conclusions:* MDx were more sensitive than culture, as confirmed by FISH. FISH only identifies bacteria that are embedded or infiltrated within the tissue and is thus not susceptible to contamination. Not all suspected cases of septic arthritis contain bacteria, but a significant percent of patients with OA, and no signs of infection, have FISH-confirmed bacterial biofilms present in the knee.

Keywords: molecular diagnostics, septic arthritis, osteoarthritis, bacterial infection, fluorescent *in situ* hybridization

Introduction

Molecular diagnostics (MDx) have been available for the detection of infections for more than 20 years (Ehrlich and Greenberg, 1994; Marshall *et al.*, 1997) and have routinely been demonstrated to be superior to culture techniques (Ehrlich and Greenberg, 1994; Aul *et al.*, 1998); however, their adoption in clinical medicine has been limited by time and cost constraints (Zmistowski *et al.*, 2014) as well as by the lack of common diagnostic criteria and the lack of

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single-format tests that provide for unbiased panel-based and pan-domain testing (Ehrlich, 1996; Hall-Stoodley *et al.*, 2012; Zmistowski *et al.*, 2014). As MDx technology improves and cost barriers decrease, implementation of these tools is becoming more widespread. The current generation of computationally based nucleic acid tests has the ability to provide greater diagnostic accuracy more rapidly than culture-based methods while doing so more rapidly and efficiently (Kathju *et al.*, 2009; Tuttle *et al.*, 2011; Yun *et al.*, 2012; Boase *et al.*, 2013; Vento *et al.*, 2013; Nickel *et al.*, 2015). In particular, the polymerase chain reaction–electrospray ionization–time-of-flight mass spectrometry (PCR–ESI–TOF–MS) platform has shown promise in diagnosing orthopedic infections (Stoodley *et al.*, 2008, 2011a; Kathju *et al.*, 2010; Costerton *et al.*, 2011; Gallo *et al.*, 2011; Ehrlich *et al.*, 2012; Jacovides *et al.*, 2012; Howe *et al.*, 2013; Palmer *et al.*, 2014).

Rapid bacterial detection in sterile body fluid specimens is vitally important for many reasons. First, identifying whether or not bacteria are actually present provides important differential diagnostic information. Second, identifying the taxon of the invading pathogen provides information critical for aiding in identifying the likely source, that is, community acquired versus nosocomial. Third, identification of antibiotic resistance provides for the development of targeted antibiotic treatment regimens. Current microbiological techniques can take anywhere from 2 to 21 days to diagnose the presence of bacteria and provide an antibiogram. This can lead to prolonged treatment with broad-spectrum antibiotics or a lag in antibiotic administration until the cultures turn positive if infection is not suspected. MDx techniques have the ability to provide all of this information within hours, not days, as the genes for antibiotic resistance can be detected as well as the pathogens themselves (Ecker *et al.*, 2008; Ehrlich *et al.*, 2012).

Rapid MDx can play an important role in orthopedics as many bacterial infections, particularly chronic infections, are either difficult to detect using standard culture or are occult and do not provide the clinical signs and symptoms usually associated with acute infection (Ehrlich *et al.*, 2012, 2014). One of the primary means by which bacteria persist in the human body is through the formation of complex differentiated bacterial communities, termed biofilms, which are recalcitrant to standard microbial culture and antibiotic treatments (Kathju *et al.*, 2009; Ehrlich *et al.*, 2010; Costerton *et al.*, 2011; Hall-Stoodley *et al.*, 2012; Boase *et al.*, 2013; Nickel *et al.*, 2015). Thus, culture-based techniques have high rates of false negatives compared with modern MDx techniques (Stoodley *et al.*, 2008, 2011a; Kathju *et al.*, 2009, 2010; Costerton *et al.*, 2011; Gallo *et al.*, 2011; Tuttle *et al.*, 2011; Ehrlich *et al.*, 2012; Jacovides *et al.*, 2012; Kreft *et al.*, 2012; Boase *et al.*, 2013; Howe *et al.*, 2013; Vento *et al.*, 2013; Palmer *et al.*, 2014; Nickel *et al.*, 2015).

In orthopedics, synovial fluid analyses provide a great deal of information with regard to the biology of the joint, and useful clinical treatment algorithms have been implemented based on the findings. The likelihood of diagnosing septic arthritis increases with elevation in white blood count, percent neutrophils, and protein level, as well as a decrease in glucose level. Definitive diagnoses have classically relied on the presence of a positive culture and/or Gram stain. Unfortunately, the sensitivities of culture and Gram stain are very low (Pascual and Jovaní, 2005; Brannan and Jerrard, 2006; Courtney and Doherty, 2013).

In this study, we compared the sensitivity of the PCR–ESI–TOF–MS platform (Ibis Universal Biosensor; Abbott Molecular, Carlsbad, CA) (Ecker *et al.*, 2008) with traditional microbial cultural techniques to determine its possible role for rapid diagnoses of native joint septic arthritis and occult chronic bacterial infection associated with osteoarthritis (OA).

Patients and Methods

Patient populations

Ethical approval was obtained from the Allegheny Singer Research Institute Institutional Review Board. External funding was obtained from The Pittsburgh Foundation. Two cohorts of patients were analyzed, all of whom were admitted to a single level 1 teaching hospital: the first cohort consisted of patients requiring an orthopedic consultation for knee effusion who were suspected of septic arthritis requiring aspiration (44 pts); the second cohort consisted of patients undergoing elective primary total knee arthroplasty (21 pts) for OA. Informed consent was obtained before specimen acquisition from all patients. Exclusion criteria for the septic arthritis cohort included patients who were unable to provide informed consent, pregnancy, children under the age of 18, or a dry aspirate. Exclusion criteria for the OA cohort included a history of septic arthritis or a dry aspirate.

Clinical specimens

All samples for the septic arthritis cohort were collected during diagnostic aspiration at the bedside by a single orthopedic surgeon (M.P.P.) to ensure consistency in specimen acquisition. The knee area was prepped with betadine and an 18 G needle was inserted using a standard superior-lateral approach. The aspirate was divided into two aliquots. The first aliquot was processed for routine laboratories: Gram stain, bacterial culturing, cell count with white blood cell differential, crystal analysis, protein levels, and glucose levels. The second aliquot was analyzed for the composition and diversity of microbes using the PCR–ESI–TOF–MS MDx (Ecker *et al.*, 2008) and/or for direct microscopic visualization of bacterial biofilms by 16S rRNA fluorescence *in situ* hybridization (FISH) using species- or genus-specific probe sets (Nistico *et al.*, 2009, 2011).

All samples obtained from the OA cohort were collected by a single orthopedic surgeon (M.P.P.) in the operating room under sterile conditions after skin incision, using a sterile 18 G needle inserted using an antero-lateral approach, but before antibiotic administration and arthrotomy. Aspirated fluid, collected using sterile technique, was analyzed for the composition and diversity of microbes using MDx and/or for direct microscopic visualization of bacterial biofilms by FISH using species- or genus-specific probe sets.

Cultures and Gram stain

Routine microbiological cultures were prepared by dipping a sterile swab into the aspirate and inoculating blood agar plates (BAP), chocolate agar plates (CHOC), MacConkey plates (MAC), and Columbia colistin-nalidixic acid agar. The agar plates were then incubated in 5% CO_2 at 35°C. A second set of plates were inoculated after a blind broth subculture in thioglycolate media (THIO). The BAP and CHOC were incubated in 5% CO_2 at 35°C, and the MAC and THIO

were incubated in the aerobic incubator at 35°C. If growth was seen on plates after the first 24 h, then the sample was reincubated on plates and in broth for an additional 24 h. After day 2, if no growth was seen in broth or plates, the plates were discarded and reported as no growth day 2 and the THIO was placed in the rack for an additional 3 days. All significant isolates were reported on day 2, and any relevant plates were saved until day 5. If there was no growth on the plates, but growth in THIO, the THIO was Gram stained and subcultured on appropriate plates based upon the findings. The THIO was examined every 24 h until 5 days, and if no growth was found, no growth day 5 was reported. All plates were followed until day 5 and then disposed.

A Gram stain from the original aspirate was prepared by rolling a second swab on a glass slide to make a thin film. All slides made for Gram stain were examined for cells and bacteria under an oil immersion objective. Any polymorphonuclear leukocytes and mononuclear cells were reported. Any bacterial organisms seen were reported and quantified numerically.

Crystal analyses for gout and pseudogout

Synovial fluid aspirate samples from all patients in both cohorts were analyzed for crystals by placing a drop of the synovial fluid sample on a glass slide and viewing under a high-powered microscope. The presence of rhomboid-shaped positively birefringent crystals was recorded as (+) for calcium pyrophosphate deposition (pseudogout). The presence of needle-shaped negatively birefringent crystals was recorded as (+) for monosodium urate crystals (gout). The lack of crystals was recorded as negative result.

Aspirate acquisition and preparation for MDx

Immediately after acquisition, the specimens were aliquoted and the aliquot for MDx was stored at −80°C. A second aliquot for bacterial 16S rRNA FISH was fixed with fresh 4% paraformaldehyde (PFA) and incubated for 2–4 h at 4°C. After the PFA incubation, the specimen was spun down and the supernatant removed. This process was repeated twice. Finally the samples were resuspended in 50% ethanol–phosphate-buffered saline solution and stored at −20°C before the 16S FISH evaluation.

MDx BAC assay for eubacterial detection and species identification using the Ibis T5000 platform

In summary, total DNA was extracted from aspirates and the bacterial DNAs were amplified by PCR (7) using the 17 primer pair BAC system developed by Ibis (Ecker *et al.*, 2008), and the individual amplicons were weighed using the Ibis T5000 platform. The species identities of the amplicons were then revealed using a database containing base composition data on virtually all bacterial species sequenced to date.

For each set of MDx analyses, multiple (two or three) negative controls were included that were processed identically to the clinical specimens using all of the same processing reagents and materials, except that no clinical material was added. Any bacterial species identified in the negative controls that also were detected in any of the clinical specimens were not included in the results. Although we did identify some organisms previously recognized as low-level contaminants in some of the commercial reagents, we did not identify any known pathogens in these controls. In addition, processing of clinical specimens and reaction setups were conducted in laboratories that were both physically and heating ventilation and air conditioning isolated from the amplification and analysis laboratories to minimize any risk of end product carryover contamination (Ehrlich and Greenberg, 1994). For all Ibis analyses, 1 mL of aspirate was centrifuged at 10,000 rpm × 3 min, then 900 μL of supernatant was removed leaving the bacteria in 100 μL. Two hundred seventy microliters of ATL lysis buffer (Qiagen, Germantown, MD; cat# 19076) and 30 μL of proteinase K (Qiagen; cat# 19131) were then added. Samples were incubated at 56°C for 1 h for proteolysis. One hundred microliters of a mixture containing 50 μl each of 0.1- and 0.7-mm Zirconia beads (Biospec; cat# 11079101z, 11079107zx, respectively) was added to the samples, which were then homogenized for 10 min at 25 Hz using a Qiagen Tissuelyser. Nucleic acids from the lysed samples were then extracted using the Qiagen DNAeasy Tissue kit (Qiagen; cat# 69506). Ten microliters of each sample was loaded per well onto the Ibis BAC detection PCR plate (Abbott Molecular; cat# PN 05 N13-01). The BAC detection plate is a 96-well plate, which contains 17 primer pairs per assay that survey all bacterial organisms by using omnipresent loci (e.g., 16S rDNA sequences); phylum/class/order-specific loci; and some are targeted to specific pathogens of interest (e.g., the *Staphylococcus*-specific tufB gene). The system also detects the presence of several key antibiotic resistance markers: van A and van B (vancomycin resistance) in *Enterococcus* species, KPC (carbapenem resistance) in gram-negative bacteria, and mec A (methicillin resistance) in Staphylococcal species. An internal calibrant of synthetic nucleic acid template is also included in each assay, controlling for false negatives (e.g., from PCR inhibitors) and enabling a semiquantitative analysis of the amount of template DNA present. PCR amplifications were carried out as per Ecker *et al.* (2008) (Courtney and Doherty, 2013) and the PCR products were then desalted in a 96-well plate format and sequentially electrosprayed into a TOF mass spectrometer. The spectral signals were processed to determine the masses of each of the PCR products present with sufficient accuracy that the nucleotide base composition of each amplicon could be unambiguously deduced. Using combined base compositions from multiple PCRs, the identities of the pathogens and a semiquantitative determination of their relative concentrations in the starting samples were established by using a proprietary algorithm to interface with the Ibis database of known organisms (Abbott Molecular).

Fluorescent in situ *hybridization*

Specimens with discordant cultural and Ibis MDx results were further analyzed by FISH, which was performed as described by Nistico *et al.* (2009, 2011), in an attempt to confirm the positive MDx results in the cases of MDx positive/culture negative or to adjudicate in cases where the two techniques reported different positive results. Briefly, fixed aspirates were attached to gelatin-coated Shandon Multispot microscope slides (Thermo Electron Corporation, Waltham, MA). When detecting gram-positive bacteria by FISH, a solution of 0.1 mg/mL lysozyme (Sigma) in 0.1 M Tris HCl pH 7.5 and 0.05 M Na_2EDTA was added to the specimens and incubated at 37°C for 3 h as an additional permeabilization step. Fixed permeabilized samples were then dehydrated in

Table 1. 16S Ribosomal Ribonucleic Acid Fluorescent *in Situ* Hybridization Probe, DNA Sequence, and Associated Bacterial Target

Probe	*16S sequence*	*Target*
Eub338	GCTGCCTCCCGTAGGAGT	All bacteria
NONEUB338	ACTCCTACGGGAGGCAGC	Nonsense sequence
Sta	TCCTCCATATCTCTGCGC	*Staphylococcus* sp.
Str	CACTCTCCCCTTCTGCAC	*Streptococcus* sp.
Sau	GAAGCAAGCTTCTCGTCCG	*Staphylococcus aureus*
PAC 16S 598	GCC CCA AGA TTA CAC TTC CG	*Propionibacterium acnes*
ENF 191	GAAAGCGCCTTTCACTCTTATGC	*Enterococcus faecalis*

an ethanol series of 80% and 100% for 3 min each and FISH was performed with fluorescently tagged 16S rRNA oligonucleotide probes. The pan-eubacterial probe (EUB338) was used as a positive control. In addition, species-specific and genus-specific probes were chosen/designed to detect the following bacteria: (1) all; (2) *Staphylococcus* sp.; (3) *Staphylococcus aureus*; (4) *Streptococci* sp., (5) *Propionibacterium acnes*; or (6) *Enterococcus faecalis* (Integrated DNA Technologies, Inc., Coralville, IA) (Table 1). All probes were conjugated with one or the other of the sulfoindocyanine dyes, Cy3 or Cy5. Eubacterial (EU338) and nonsense probes (NONEUB338) were used as positive and negative controls, respectively. Each sample was incubated with probe-specific formamide and salt concentrations and then immersed in washing buffer with the probe-specific salt concentration. Samples were rinsed in sterile MilliQ water and observed with confocal laser scanning microscopy (CLSM).

Confocal laser scanning microscopy

CLSM imaging was performed as described previously (Nistico *et al.*, 2009, 2011), Briefly, after staining, the samples on the slide were imaged with a Leica DM RXE microscope attached to a TCS SP2 AOBS confocal system (Leica Microsystems; Exton, PA) using either a 40× (numerical aperture [NA] 1.25) or a 100× (NA 1.4) oil immersion lens.

Statistics

The difference in bacterial detection rates between standard cultural methods and the Ibis MDx was tested by McNemar's test, with the null hypothesis that the probability of a sample being culture negative, but MDx positive, is equal to the probability of a sample being culture positive, but MDx negative. The exact 2 × 2 package in statistical software R was used to calculate an exact *p*-value for McNemar's test (R Core Team, 2014; Fay, 2010). The chi-squared test was used to test for the association between positive detection by MDx and clinical diagnosis of septic arthritis as well as between positive detection by culture method and clinical diagnosis of septic arthritis. All graphical displays describing the distribution of bacteria were generated in graphics package of R (R Core Team, 2002).

Results

Between October 2010 and January 2013, 65 consecutive synovial fluid aspirate samples from patients consenting to participate in the study were analyzed. There were 44 from the septic arthritis patients and 21 OA patients. Patients included in the septic arthritis cohort had all been admitted to the hospital and presented with at least one symptom/sign for septic arthritis of a native knee, including effusion, pain, redness, fever, bacteremia, and elevated blood markers (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], and white blood cell [WBC]) (Table 2). Bacteria were detected by the PCR–ESI–TOF–MS (MDx) in 22/44 (50%) of the suspected septic arthritis samples and 6/21 (29%) of the OA samples (Fig. 1). Culture detected only *S. aureus* in 6/44 septic arthritis samples and no bacteria in any OA cases. Thus, the MDx method detected bacteria in 28/65 (43%) total specimens, whereas standard microbial culture methods detected bacteria in 6/65 (9.2%) total specimens. We found the probability of culture negative, but MDx positive, was greater than the probability of culture positive, but MDx negative (p-value <0.0001); this suggests that MDx are more likely to detect the bacterial presence than standard cultural methods. Neither the MDx nor culture produced results that were significantly correlated with the physicians' suspicions of septic arthritis ($p = 0.173$ and $p = 0.186$, respectively).

MDx indicated that *S. aureus* was much more highly prevalent in the septic arthritis population 10/44 (22.7%) compared with the OA group 0/21 (0%). The next most frequent bacterium detected was *P. acnes*, present in two septic arthritis samples and two OA samples. Polymicrobial infections were identified in three specimens, two from the septic arthritis cohort and one from the OA group (Fig. 2).

Five of the six *S. aureus* culture-positive samples were also *S. aureus* positive by MDx. The only patient who showed a discrepancy in detection was positive for *Streptococcus agalactiae* by MDx (Table 3). The detection of bacteria in a significantly higher percentage of cases in both patient cohorts by MDx compared with culture prompted the use of a confirmatory method to validate the discrepant MDx findings. Toward this end, FISH was performed on all MDx-positive/culture-negative specimens with one exception due to insufficient specimen. The FISH analyses confirmed the MDx result for 19/21 (90.5%) MDx-positive/culture-negative specimens (Table 3, blue and green, respectively, and Fig. 3A). Four of the six samples that were positive by both MDx and culture were also subjected to FISH and CLSM-based visualization; as expected, these specimens were also FISH positive for *S. aureus* (Table 3, subset of purple; Fig. 3B). Importantly, for the specimen (NK1025) with discrepant positive culture and positive MDx results, the FISH analyses performed independently with probes for both species supported the MDx finding of *Streptococcus agalactiae* and not *S. aureus*, supporting the conclusion that the culture was contaminated.

Utilizing 16S FISH as the gold standard, it demonstrates a specificity of 93% (26/28) for the PCR–ESI–TOF–MS

Table 2. Clinical Characteristics of Study Patients (Added in Revision)

Study #	*Dx*	*WBC*	*ESR*	*CRP*	*SYN WBC*	*% PMN*	*Protein*	*Glu*	*Crystal*	*Cx*	*Ibis*
NK1002	SA	7	NA	NA	12,750	83	5.1	70	Neg		*Sa*
NK1005	SA	19.6	>140	26.7		not enough fluid to send for analysis				MRSA	MRSA
NK1006	SA	8.2	NA	NA	1250	95	2.7	122	Gout		Neg
NK1007	SA	9.9	67	8.3	2425	70	4	119	Gout		*Sa*
NK1008	SA	13.7	3	2.1	3600	94	3.7	57	Neg	*Sa*	Sa
NK1009	SA	12.8	30	6.7	50	54	1.3	89	Neg		Neg
NK1010	SA	NA	NA	NA	8750	4	4.7	29	Neg		Neg
NK1011	SA	7.6	79	21.7	36,000	75	6.4	89	Neg		*Se*
NK1012	SA	9.5	74	15.7	35,500	86	6.9	146	Neg		Neg
NK1013	SA	9.2	4.6	62	6150	91	6.2	218	Neg		Neg
NK1014	SA	8	78	10.5	6120	89	3.7	82	Neg		Neg
NK1015	SA	11.3	NA	26	6	55	0.3	128	Neg		*Sa*
NK1016	SA	12.6	>140	26.1	165,000	98	5.6	<2	Neg		Neg
NK1017	SA	9.2	21	5.9	18,500	90	5.1	55	Gout		*Sa*
NK1023	SA	7.4	10	20.8	16,000	99	3.3	119	Gout		*Pa*
NK1025	SA	10.2	>140	29.9	76,000	95	5.3	27	Neg	MRSA	*Strep ag*
NK1026	SA	3.9	NA	NA	300,000	57	5.2	80	Neg		*Neg*
NK1030	SA	7.3	NA	NA	4600	86	3.1	195	Neg		*Ef*
NK1031	SA	1.2	NA	NA	4500	70	NA	NA	Neg		*Neg*
NK1035	SA	8.1	18	7.8	2333	87	2.6	162	Neg		MRSA
NK1036	SA	23.3	33	21.5	210,000	73	4.3	2	Gout	MRSA	MRSA
NK1044	SA	8.2	92	19.6	13,000	87	3.8	93	Gout		*Sh*
NK1053	SA	18.7	68	37.1	20,000	81	4.5	45	Neg	MRSA	MRSA
NK1055	SA	7.5	4	1.2	1100	94	2.5	162	Gout		*Sc*
NK1058	SA	4.9	55	<0.3	23,000	95	2.6	131	Neg		*ND*
NK1059	SA	10.1	NA	NA	12,000	90	3.6	62	Neg		*Neg*
NK1064	SA	8.4	34	12.2	58,500	90	2	140	Gout		*Neg*
NK1065	SA	23.7	84	31.4	29,500	95	3.9	114	Neg		*Neg*
NK1066	SA	NA	NA	NA	9300	77	4	70	Neg		*Lm*
NK1069	SA	6.2	NA	NA	14,750	77	3.9	212	Gout		*Neg*
NK1070	SA	12.7	63	13.5	90,000	86	5.5	<2	Neg	*S.* sp.	*Se and Sl*
NK1071	SA	4.4	NA	NA	NA	NA	2.2	88	Neg		*Neg*
NK1072	SA	6.5	32	1.8	550	46	NA	NA	NA		*Neg*
NK1073	SA	6.4	54	5.6	1600	77	7.5	31	Neg		*Neg*
NK1075	SA	NA	NA	NA	300	8	4.1	16	Neg		*Neg*
NK1076	SA	8.8	66	10.6	15,000	84	4.2	183	Neg		*Neg*
NK1077	SA	8.8	66	10.6	14,000	86	3.8	168	Neg		*Bt*
NK1078	SA	9.9	59	5.1	11,000	91	4.4	148	Neg		*Neg*
NK1079	SA	12.5	18	12.8	41,000	84	3.6	492	Gout		*Neg*
NK1080	SA	5.9	21	2.7	29,000	81	4.6	130	CPPD		*Strep py*
NK1081	SA	21	60	25.8	41,500	93	4.5	109	Gout		*Nm*
NK1082	SA	21	60	25.8	28,875	95	3.7	97	Gout		*Nm*
NK1083	SA	13	NA	NA	26,250	96	4.3	153	Gout		*Neg*
NK1084	SA	8	64	26.2	29,750	91	2.2	134	Gout		*Neg*
NK1085	SA	6	56	3.8	48,125	63	2.4	100	Neg		*Pa*
NK1086	SA	6.6	17	2.8	5778	48	3.6	99	Neg		*Neg*
NK1061	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1062	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1067	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1068	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1087	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1088	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1089	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1090	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Se*
NK1091	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1092	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Pa*
NK1093	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1094	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Sc*
NK1095	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1096	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1097	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1098	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1099	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Ch*
NK1100	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Nm*
NK1101	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Pa*
NK1102	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*
NK1103	OA	ND	ND	ND	ND	ND	ND	ND	ND		*ND*
NK1105	OA	ND	ND	ND	ND	ND	ND	ND	ND		*Neg*

Study #, study number; Dx, clinical diagnosis or suspected clinical diagnosis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SYN WBC, synovial white blood cell count; % PMN, percent polymorphonuclear leukocytes; Protein, protein concentration; glucose, glucose concentration; crystals, type of crystal if any found; Cx, positive microbiological culture result from synovial fluid; MRSA, methicillin-resistant *Staphylococcus aureus*; SA, septic arthritis; OA, osteoarthritis; NA, not assessed; ND, not determined based on diagnosis of osteoarthritis; neg, negative for any crystals; gout, positive for monosodium urate crystals; *Sa*, *Staphylococcus aureus*; *Se*, *Staphylococcus epidermidis*; *Pa*, *Propionibacterium acnes*; *Strep ag*, *Streptococcus agalactiae*; *Ef*, *Enterococcus faecalis*; *SH*, *Staphylococcus hominis*; *Sc*, *Staphylococcus capitas/caprae*; *Lm*, *Listeria monocytogenes*; *S.* sp., *Staphylococcus* species; *Sl*, *Staphylococcus lugudensis*; *Bt*, *Bacillus thuringiensis*; *Strep py Streptococcus pyogenes*; *Nm*, *Neisseria meningitides*; *Ch*, *Campylobacter hyointestinalis.*

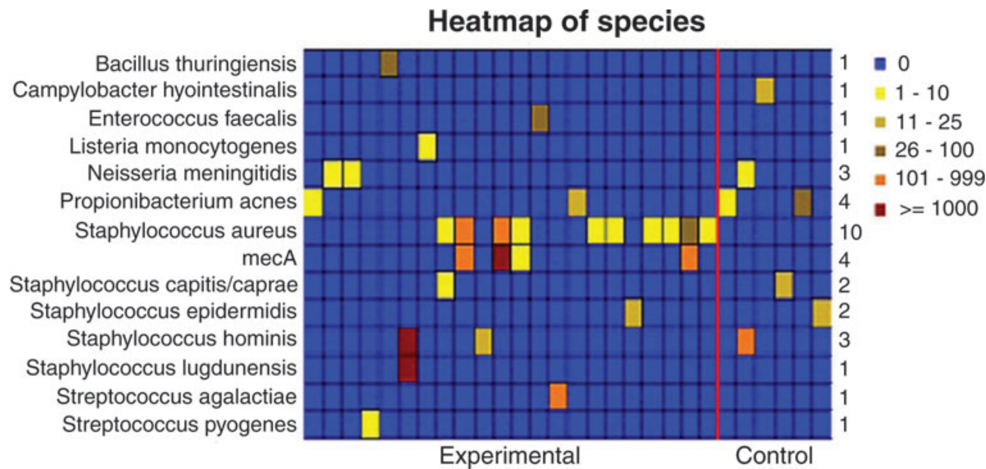

FIG. 1. Microbial composition of synovial fluid using polymerase chain reaction–electrospray ionization–time-of-flight mass spectrometry (IBIS) for molecular diagnosis represented as a heat map. *Colored squares* represent a detection of species (*row*) in a patient (*column*) with different *colors* associated with the number of genomes per well; in addition, the presence of methicillin resistance genes (mecA) is also denoted. The *red line* denotes the separation of experimental (*left*) and control (*right*) sets. The numbers on the *right* margin indicate the frequency of species in all samples. Experimental refers to the patient cohort with suspected septic arthritis, and control refers to the patient cohort with osteoarthritis.

MDx for bacterial detection in synovial fluid aspirates regardless of clinical diagnosis. Looking solely at the septic arthritis cohort, MDx had a specificity of 91% (21/23). It was not possible to assess sensitivity since all, but two, of the specimens examined by FISH were chosen based on their discordant MDx-positive and culture-negative results.

There was no correlation between the clinical laboratory parameters (cell count with white blood cell differential, presence or absence of crystals (Fig. 4), protein levels, and glucose levels) and MDx findings. These findings suggest that while these parameters may be useful to detect host responses to highly virulent pathogens, they do not correlate with the presence or absence of microbes generally and chronic pathogens in particular.

Discussion

Rapid detection and swift targeted treatment of septic arthritis are critically important to preserve articular cartilage (Mathews *et al.*, 2010). It is also important to prevent invasive spread of the infection to other musculoskeletal locations as well as to reduce the risk of systemic spread. Current diagnostic methods for septic arthritis rely on secondary signs of infection (WBC, CRP, ESR, synovial fluid analysis) and clinical judgment to initiate treatment while waiting for 24–120 h or more for culture results and antibiotic susceptibility profiles to be used for the rational selection of antimicrobial therapies (Pascual and Jovaní, 2005).

PCR–ESI–TOF–MS has the capability of providing both bacterial identification at the species level and antibiotic

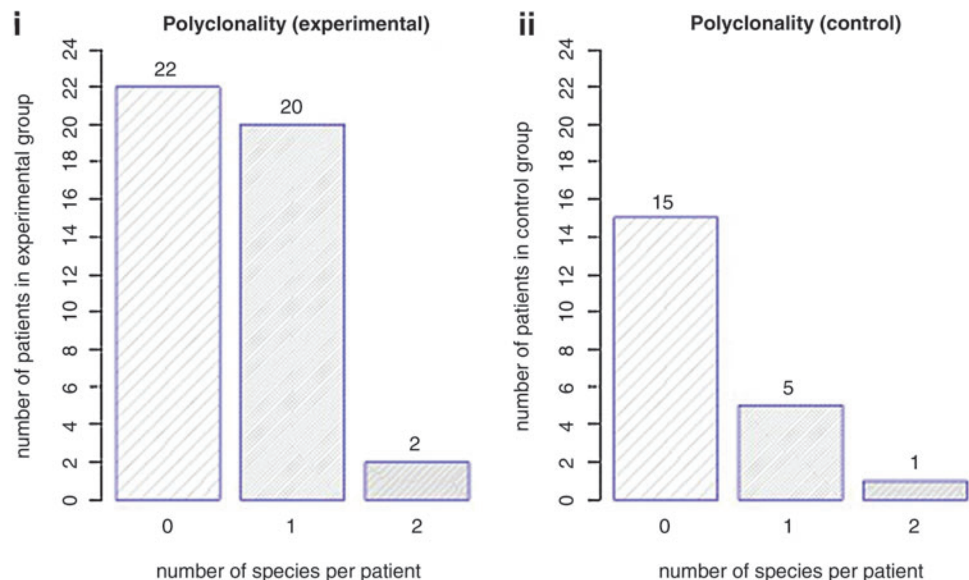

FIG. 2. Distribution of bacteria identified by MDx per encounter. **(i)** Experimental (septic arthritis) group ($n=44$) and **(ii)** the control (osteoarthritis) group ($n=21$). MDx, molecular diagnostics.

Table 3. Results of Polymerase Chain Reaction–Electrospray Ionization–Time-Of-Flight Mass Spectrometry (IBIS), Fluorescent *in Situ* Hybridization, and Cultures for Each Patient Encounter that was IBIS or Fluorescent *in Situ* Hybridization Positive

ID	Group	IBIS	FISH (positive unless noted)	Culture	Ibis/Culture Agreement
NK1077	Septic arthritis	*Bacillus thuringiensis*	EUB	negative	Disagree
NK1099	Osteoarthritis	*Campylobacter hyointestinalis*	EUB	negative	Disagree
NK1030	Septic arthritis	*Enterococcus faecalis*	*E. faecalis*	negative	Disagree
NK1066	Septic arthritis	*Listeria monocytogenes*	EUB	negative	Disagree
NK1031	Septic arthritis	negative	EUB (-)	negative	Agree
NK1014	Septic arthritis	Negative	EUB (-)	negative	Agree
NK1006	Septic arthritis	Considered Negative / very low *S. epidermidis* copy number	Staphylococcus sp. (very low cell count)	negative	Disagree
NK1081	Septic arthritis	*Neisseria meningitidis*	EUB	negative	Disagree
NK1082	Septic arthritis	*Neisseria meningitidis*	EUB	negative	Disagree
NK1100	Osteoarthritis	*Neisseria meningitidis* *Staphylococcus hominis*	EUB	negative	Disagree
NK1085	Septic arthritis	*Propionibacterium acnes*	insufficient sample	negative	Disagree
NK1023	Septic arthritis	*Propionibacterium acnes*	*P. acnes*	negative	Disagree
NK1101	Osteoarthritis	*Propionibacterium acnes*	*P. acnes*	negative	Disagree
NK1092	Osteoarthritis	*Propionibacterium acnes*	*P. acnes*	negative	Disagree
NK1002	Septic arthritis	*Staphylococcus aureus*	*S. aureus*	negative	Disagree
NK1015	Septic arthritis	*Staphylococcus aureus*	*S. aureus*	negative	Disagree
NK1007	Septic arthritis	*Staphylococcus aureus*	*S. aureus (-)*	negative	not confirmed by FISH
NK1017	Septic arthritis	*Staphylococcus aureus*	*Staphylococcus sp.*	negative	Disagree
NK1035	Septic arthritis	*Staphylococcus aureus*, mecA	*S. aureus*	negative	Disagree
NK1055	Septic arthritis	*Staphylococcus aureus*, *Staphylococcus capitis/caprae*	*S. aureus*	negative	Disagree
NK1094	Osteoarthritis	*Staphylococcus capitis/caprae*	*Staphylococcus sp.*	negative	Disagree

(*continued*)

Table 3. (Continued)

ID	Group	IBIS	FISH (positive unless noted)	Culture	Ibis/Culture Agreement
NK1011	Septic arthritis	*Staphylococcus epidermidis*	*Staphylococcus sp (-).*	negative	not confirmed by FISH
NK1090	Osteoarthritis	*Staphylococcus epidermidis*	*Staphylococcus sp.*	negative	Disagree
NK1044	Septic arthritis	*Staphylococcus hominis*	*Staphylococcus sp.*	negative	Disagree
NK1080	Septic arthritis	*Streptococcus pyogenes*	*Streptococcus sp.*	negative	Disagree
NK1005	Septic arthritis	*Staphylococcus aureus*, mecA	*S. aureus*	S. aureus (MRSA)	Agree
NK1036	Septic arthritis	*Staphylococcus aureus*, mecA	ND	S. aureus (MRSA)	Agree
NK1053	Septic arthritis	*Staphylococcus aureus*, mecA	ND	S. aureus (MRSA)	Agree
NK1025	Septic arthritis	*Streptococcus agalactiae*	*Streptococcus sp.*	S. aureus (MRSA)	Different Organism
NK1008	Septic arthritis	*Staphylococcus aureus*	*S. aureus*	staph aureus	Agree
NK1070	Septic arthritis	*Staphylococcus hominis* *Staphylococcus lugdunensis*	*Staphylococcus sp.*	Staph species	Agree

Blue background highlighting indicates discordance between microbial culture and PCR-based DNA diagnostics (MDx) where the FISH results support the MDx result; *yellow* background highlighting indicates concordance of negative findings between microbial culture and MDx; *green* background highlighting indicates discordance between microbial culture and PCR-based DNA diagnostics (MDx) where the FISH results support the culture result; *purple* background highlighting indicates concordance of positive bacterial findings between microbial culture and MDx.

EUB, Pan-domain eubacterial probe (will hybridize with any bacterial 16S sequence); ND, not determined; PCR, polymerase chain reaction; FISH, fluorescent *in situ* hybridization; MDx, molecular diagnostics.

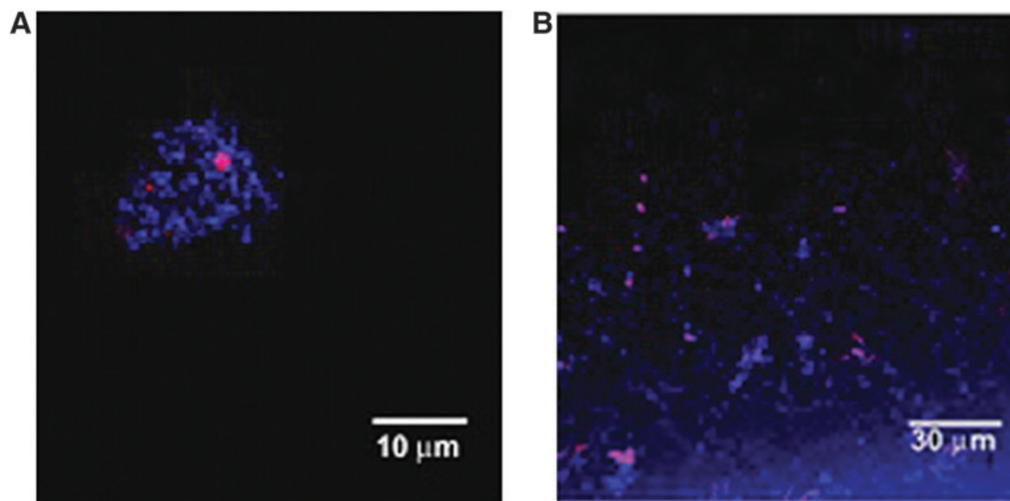

FIG. 3. Confocal microscopic images of biofilm bacteria labeled using FISH. The bacterial cells are visualized as *pink* in a *blue* (reflected light) background representing the synovial tissue taken from arthroscopic debridement and irrigation of two cases. **(A)** Sample NK1025 (osteoarthritis) stained with a *Streptococcus* sp.-specific FISH probe (*pink*). This specimen was MDx positive for *Streptococcus agalactiae*, but culture positive for *Staphylococcus aureus*. FISH performed on this specimen for *S. aureus* was negative. **(B)** Sample NK1092 (osteoarthritis) stained with *Propionibacterium acnes*-specific FISH probe (*pink*). FISH, fluorescent *in situ* hybridization.

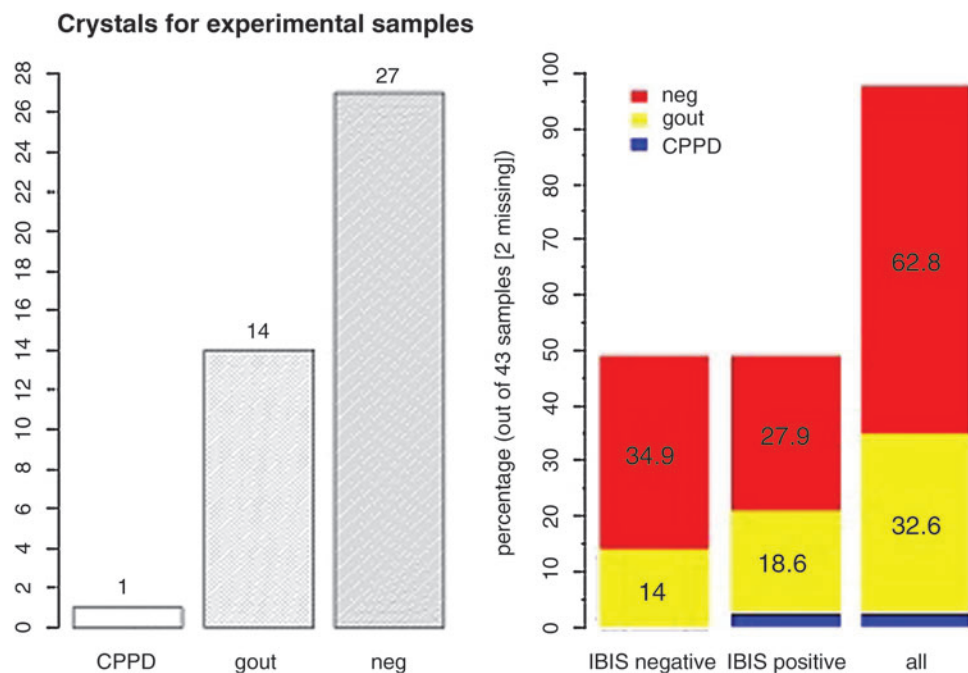

FIG. 4. Comparison of clinical laboratory findings for gout and pseudogout (CPPD) crystals with MDx testing. *Left panel*: Crystal analysis of experimental (septic arthritis) group ($n = 42$) for CPPD and gout (uric acid); *right panel*: No correlation was found between CPPD or gout and bacterial identification on MDx. CPPD, calcium pyrophosphate dehydrate.

sensitivity predictions within 6 h of sample collection (Costerton *et al.*, 2011; Stoodley *et al.*, 2011b). This improved efficiency could significantly reduce the potential complications of prolonged broad-spectrum antibiotic usage such as the development of increased antimicrobial resistance, *Clostridium difficile* colitis, and kidney damage, while significantly decreasing hospital costs.

In our study, all of the culture-positive cases were identified as *S. aureus*, consistent with historical findings (Mathews *et al.*, 2010). The MDx data confirmed the cultural findings in the majority of cases, which is also consistent with previous reports (Palmer *et al.*, 2011, 2014). In the one culture-positive/MDx-positive species discrepant case (NK1025) the MDx finding of *Streptococcus agalactiae* was validated over the culture results using 16S rRNA FISH as an independent confirmatory method. A possible explanation for this discrepancy is that the patient was polyclonally infected and streptococci represented the vast majority of the sample such that any staphylococcal signal was below the detection threshold of MDx and FISH, alternatively the *S. aureus* finding may have resulted from contamination of the culture. The fact that positive MDx findings in the case of culture-negative cases did not always correlate with the laboratory results obtained for generic markers of inflammation is not surprising as the inflammatory response depends on the metabolic state of the bacteria (Ehrlich *et al.*, 2012, 2014) and the immune status of the patient. Patients were hospitalized for a variety of reasons that could elevate inflammatory markers. Inflammatory responses to staphylococcal toxins do not correlate with the bacterial titers that are reported by the PCR–ESI–TOF–MS approach.

There were two septic arthritis cases with discordant MDx and FISH results. These could either reflect sampling error when performing FISH, as bacterial biofilms are known to be geographically nonhomogeneous (Hall-Stoodley *et al.*, 2006; Ehrlich *et al.*, 2010), or represent true false-positive results from the MDx.

Twenty-eight percent (6/21) of the specimens from patients with OA undergoing primary arthroplasty also contained bacteria that were detected by both the PCR–ESI–TOF–MS and 16S FISH. Not surprisingly, and in concordance with previous findings (Jacovides *et al.*, 2012), the bacterial species identified in the OA cases were different than those identified in the septic arthritis cases and represented slow-growing or opportunistic pathogens, including *P. acnes* and coagulase-negative staphylococci; organisms that do not necessarily promote purulent and pyogenic responses in the host.

Whereas it can be argued from a clinical perspective that all MDX-positive OA cases represent false positives as there are no overt signs or symptoms of infection, it is also quite clear from a microbiological perspective that there are bacteria present. These data are consistent with our previous observations (Jacovides *et al.*, 2012; Ehrlich *et al.*, 2014) that bacteria are often present in knees of osteoarthritic patients, but it must be emphasized that the clinical significance (if any) of these findings is unknown. We have speculated that bacteria in the knee joint of OA patients may play an etiological and/or exacerbatory role in the disease process, but unless these hypotheses can be substantiated mechanistically, their finding should probably not be used to initiate any type of antimicrobial treatment. These findings are consistent with studies that have shown that bacteria can survive within synovial cells (Shirtliff and Mader, 2002). However, it should be emphasized that these findings should be interpreted very cautiously with regard to making a diagnosis of septic arthritis. It is possible, however, through chronic or subacute processes (that do not induce standard inflammatory symptomatology by the host) that these infections do contribute to the pathogenesis of OA (Ehrlich *et al.*, 2014).

The most valuable data obtained from the present study may well be the finding that $\sim$50% of the patients with clinical suspicion of septic arthritis are negative by the PCR–ESI–TOF–MS methodology. Thus, future studies based on this finding could be designed to rapidly test suspected septic

arthritis cases. Those patients who were MDx negative would be assigned to a watchful waiting status rather than treatment with broad-spectrum antibiotics.

One of the limitations of this study is the lack of follow-up clinical data on the OA patients with regard to their risk of subsequent periprosthetic joint infections based on their MDx status for bacterial presence. Thus, future studies should include a longitudinal component and animal models to determine the pathogenicity of the nonpurulent bacterial species identified by MDx.

In conclusion, based on its concordance with 16S bacterial FISH, the PCR–ESI–TOF–MS MDx has the potential to improve diagnostic accuracy and efficiency in the diagnosis and treatment of septic arthritis.

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Author Disclosure Statement

No competing financial interests exist.

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