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Evaluation of the CosmosID Bioinformatics Platform for Prosthetic Joint-Associated Sonicate Fluid Shotgun Metagenomic Data Analysis

Qun Yan,[a,b] Yu Mi Wi,[a,c] Matthew J. Thoendel,[d] Yash S. Raval,[a] Kerryl E. Greenwood-Quaintance,[a] Matthew P. Abdel,[e] Patricio R. Jeraldo,[f] Nicholas Chia,[f] Robin Patel[a,d]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[b]Department of Clinical Laboratory, Xiangya Hospital of Central South University, Changsha, Hunan, China
[c]Division of Infectious Diseases, Department of Medicine, Samsung Changwon Hospital, Sungkyunkwan University, Changwon, South Korea
[d]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA
[e]Department of Orthopedic Surgery, Mayo Clinic, Rochester, Minnesota, USA
[f]Department of Surgery, Mayo Clinic, Rochester, Minnesota, USA

ABSTRACT We previously demonstrated that shotgun metagenomic sequencing can detect bacteria in sonicate fluid, providing a diagnosis of prosthetic joint infection (PJI). A limitation of the approach that we used is that data analysis was time-consuming and specialized bioinformatics expertise was required, both of which are barriers to routine clinical use. Fortunately, automated commercial analytic platforms that can interpret shotgun metagenomic data are emerging. In this study, we evaluated the CosmosID bioinformatics platform using shotgun metagenomic sequencing data derived from 408 sonicate fluid samples from our prior study with the goal of evaluating the platform *vis-à-vis* bacterial detection and antibiotic resistance gene detection for predicting staphylococcal antibacterial susceptibility. Samples were divided into a derivation set and a validation set, each consisting of 204 samples; results from the derivation set were used to establish cutoffs, which were then tested in the validation set for identifying pathogens and predicting staphylococcal antibacterial resistance. Metagenomic analysis detected bacteria in 94.8% (109/115) of sonicate fluid culture-positive PJIs and 37.8% (37/98) of sonicate fluid culture-negative PJIs. Metagenomic analysis showed sensitivities ranging from 65.7 to 85.0% for predicting staphylococcal antibacterial resistance. In conclusion, the CosmosID platform has the potential to provide fast, reliable bacterial detection and identification from metagenomic shotgun sequencing data derived from sonicate fluid for the diagnosis of PJI. Strategies for metagenomic detection of antibiotic resistance genes for predicting staphylococcal antibacterial resistance need further development.

KEYWORDS metagenomics, PJI, antimicrobial resistance, prosthetic joint infection, sonicate fluid

Prosthetic joint infection (PJI) is a serious complication of arthroplasty, with an infection rate of approximately 1 and 2% for hip and knee arthroplasties, respectively (1). Conventional cultures of synovial fluid and periprosthetic tissue are commonly used for the microbiological diagnosis of PJI. Culture methods specific for PJI have been developed and shown to improve diagnostic accuracy; these include the use of blood culture bottles for periprosthetic tissue culture and implant sonication with semiquantitative culture of the resultant sonicate fluid for implant culture (2, 3). Some unusual microorganisms (e.g., mycobacteria, fungi, *Ureaplasma* species, *Mycoplasma*

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Address correspondence to Robin Patel, patel.robin@mayo.edu.

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species) which need specialized culture methods are not reliably detected by conventional aerobic and anaerobic cultures (4, 5). In a previous study, for example, we reported a PJI subject with *Mycobacterium bovis* BCG detected through periprosthetic tissue mycobacterial culture who had negative aerobic and anaerobic cultures of periprosthetic tissue and sonicate fluid (6). Specialized cultures are typically ordered only based on the clinicians' judgment of a suspected unusual infection, which may result in missed diagnoses. Broad-spectrum agnostic tools for detecting pathogens unbiasedly, including those that are difficult to culture or unculturable, are needed (7).

New massive parallel deep sequencing technologies are revolutionizing the diagnosis of infectious diseases. Whole-genome sequencing is being used on cultured isolates to identify them and determine their genetic characteristics, including their antibiotic resistance and virulence traits, as well as their clonal relatedness to other isolates (8–10). Targeted metagenomic sequencing has been applied to interrogate specific genes, and more recently, metagenomic shotgun sequencing has been developed and applied in the field of infectious diseases (11). Metagenomic shotgun sequencing is a method of sequencing all the nucleic acid in a specimen unbiasedly and then matching the sequences to those in databases to identify organisms and their genetic traits (e.g., antibiotic resistance genes and mutations). This approach has the potential to identify a variety of infectious agents, including bacteria, viruses, fungi, and protists, directly from various clinical samples, with applications being described for central nervous system specimens (12–16), respiratory tract specimens (17, 18), blood (19–22), urine (23, 24), bile (25), stool (26), synovial fluid (27), and prosthesis sonicate fluid (28–30).

We recently conducted what we believe is the largest study to date of metagenomic shotgun sequencing of sonicate fluid samples derived from prosthetic hips and knees. DNA from sonicate fluid samples was extracted after being enriched with MolYsis kits (Molzym, Bremen, Germany) for removal of human DNA. Samples were sequenced using a HiSeq 2500 platform (Illumina, San Diego, CA) in rapid run mode with paired-end reads at 250 cycles. Sequencing data were then analyzed using the Livermore Metagenomics Analysis Toolkit (LMAT) and MetaPhlAn2 tools to detect bacteria, after screening out human reads. Common contaminant genera were defined by sequencing controls and observations from previous studies. Organisms other than contaminant genera were considered positive if they made up $>$80% of the microbial reads and had $>$10,000 reads; however, multiple organisms did not need meet the criterion of $>$80% microbial reads. Organisms with $<$100 reads were considered not significant. Common contaminants or organisms with $<$10,000 reads were then interpreted using $>$10-fold the standard deviation of the average depth of coverage as a parameter to confirm contaminants, as previously described (31). The results showed metagenomic shotgun sequencing to be capable of identifying a range of PJI pathogens, including difficult-to-culture organisms; however, the detection of antibiotic resistance genes was not analyzed (31). Our study also highlighted the value of using multiple open-source analytic tools to overcome the limitation of individual approaches and of having resident bioinformatics expertise to guide interpretation. A lack of accurate automated interpretative systems for translating metagenomic sequencing data into clinical-grade reports in a timely fashion is a barrier to routine use (32). More efficient ways to analyze shotgun metagenomic sequencing data are needed; automated, high-performance bioinformatics tools will enable speed and accuracy in microbial identification, and curated databases will idealize resolution in identification, discrimination of pathogens from near neighbors, and accurate measurement of concentrations (33). To address this rapidly growing need, CosmosID (Rockville, MD) developed an automated bioinformatics platform to analyze metagenomic shotgun sequence data. Herein, we studied the CosmosID bioinformatics platform using data from our prior study (31). We focused on microorganism detection and identification and also evaluated the detection of staphylococcal resistance genes.

MATERIALS AND METHODS

Study design. Metagenomic shotgun sequencing data for sonicate fluid samples from 408 subjects (31) were analyzed using the CosmosID platform for identifying bacteria and antibiotic resistance genes. The first 204 samples were grouped as a derivation set, with the remaining 204 samples being grouped as a validation set. Bacterial detection and the antibiotic resistance genes reported from the derivation set, in the context of known results of culture and phenotypic antibacterial susceptibility testing, were used to develop an algorithm for identifying bacteria and predicting staphylococcal antibiotic resistance. The algorithm was then tested on the validation set, and the diagnostic accuracy of metagenomic sequencing was compared with that of sonicate fluid culture.

Subject characteristics. Subjects were classified as having PJI or aseptic failure using Infectious Diseases Society of America (IDSA) PJI diagnostic criteria (33), as described in our previous study (31).

Sonicate fluid culture and antibacterial susceptibility testing. Sonicate fluid culture was performed as previously described (6). Briefly, prostheses were collected and placed in sterile containers (Nalgene, Lima, OH). Sterile Ringer's solution was then added to the container. The container was vortexed for 30 s and then sonicated for 5 min (40 kHz) using an ultrasonic cleaning bath (Branson Ultrasonics, Richmond, VA), followed by additional vortexing for 30 s. The sonicate fluid was centrifuged at 4,000 rpm (3,150 $\times$ *g*) for 5 min and concentrated 1:100; 0.1 ml of the concentrated sonicate fluid was inoculated onto aerobic blood and anaerobic blood agar and incubated for 5 and 14 days, respectively. A single colony growing on a plate is equivalent to one colony per 10 ml sonicate fluid. A cutoff value of $\geq$20 CFU/10 ml was defined as positive, following the standard of care at the Mayo Clinic (34). Antibacterial susceptibility testing was done at the time of growth from sonicate fluid or periprosthetic tissue culture in the clinical microbiology laboratory at Mayo Clinic. The antibacterial agents to which *Staphylococcus* susceptibility was tested were per institutional standards. MIC values were determined by agar dilution, with the results being interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines.

Bacterial detection and staphylococcal antibiotic resistance prediction. DNA sequencing data for the 408 sonicate fluid samples from our previous study were analyzed for bacteria and antibiotic resistance genes using CosmosID's bioinformatics pipeline. Briefly, raw sequence files were uploaded to the CosmosID cloud application, with no parameters being set or modified for data upload. As previously described, the pipeline utilizes high-performance k-mer-based algorithms and curated taxonomy databases (GenBook), accessible by a cloud interface (35–37). A complete description of the k-mer-based taxonomy assignment algorithms used is provided elsewhere (35–37). Briefly, the pipeline has a first precomputation phase and a second per-sample computation (33). The phase outputs a reference microbial database to a whole-genome phylogeny tree, with sets of fixed-length k-mer fingerprints being uniquely identified with distinct nodes of the tree (33). The second computational phase searches short sequence reads against the fingerprint sets and gives fine-grain composition and relative abundance estimates at all nodes of the tree; it uses edit distance-scoring techniques to compare a target sample with a reference set (33). The first comparator finds reads with an exact match with an n-mer uniquely identified with a set of reference strains, with the second statistically scoring the entire read against the reference to verify that the read is uniquely identified with that set (33). The turnaround time for data analysis from sequence acquisition to metagenomic reports was 5 to 10 min. The reports included four variables for each bacterium or resistance gene detected: unique match frequency, unique match percentage, total matches percentage, and relative abundance. Unique match frequency is the number of unique k-mer occurrences in the queried sample and is roughly equivalent to the number of reads that match the organism. Unique match percentage is the number of unique matches divided by the number of total unique patterns for that organism in the reference database. Total matches percentage is the total number of matches (shared plus unique) divided by the total number of patterns for that organism in the reference database. Relative abundance is calculated based on the number of organism-specific k-mers and their observed frequency in the sample and then normalized to represent the abundance of each organism.

Bacterial identification interpretive algorithm development using the derivation set. Bacteria identified in cultures of sonicate fluid specimens from PJI subjects were defined as real pathogens. Data from this subset of subjects were used to preliminarily establish cutoffs for frequency, total match percentage, and relative abundance for the CosmosID analysis by manual inspection. Then, with the bacteria detected in the negative controls being defined as contaminant species, the same parameters were reevaluated and fine-tuned. Revised threshold values were then applied to the full derivation data set and adjusted to achieve optimum sensitivity and specificity.

Antibiotic resistance gene threshold using the derivation set. In the derivation set, antibiotic resistance genes from samples with *Staphylococcus* species identified by metagenomic sequencing (with or without other identified organisms) and also by sonicate fluid, synovial fluid, or periprosthetic tissue culture on which antibacterial susceptibility testing had been performed in the clinical microbiology laboratory were profiled. The genes profiled were *mecA* for prediction of methicillin resistance, *erm* for prediction of clindamycin resistance, *dfr* for prediction of trimethoprim-sulfamethoxazole resistance, and *vanA* for prediction of vancomycin resistance (8). The antibiotic resistance prediction was compared with phenotypic susceptibility testing results from the clinical microbiology laboratory. The parameter used for antibiotic resistance prediction was the antibiotic gene total matches percentage. Receiver operating characteristic (ROC) curves (Prism software, version 7.0; GraphPad, San Diego, CA) were used to set cutoff values to achieve the optimum sensitivity and specificity for predicting antibiotic resistance.

Validation of the thresholds for bacterial identification and antibiotic resistance prediction. The metagenomic bacterial detection and antibiotic resistance gene results from the 204 samples in the

TABLE 1 Characteristics of study subjects

Characteristic	Values for subjects in:	
	Derivation set ($n = 204$)	Validation set ($n = 204$)
Mean (range) age (yr)	65.2 (18–93)	65.4 (30–89)
No. (%) of male subjects	95 (46.6)	110 (53.9)
No. (%) of subjects with the following type of prosthetic joint:		
Hip	63 (30.9)	64 (31.4)
Knee	141 (69.1)	140 (68.6)
No. (%) of subjects with the presence of a sinus tract	30 (14.7)	22 (10.8)
No. (%) of subjects receiving antibiotics within 4 wk prior to surgery	56 (27.5)	79 (38.7)
No. (%) of subjects with the following preoperative laboratory findings[a]:		
White blood cell count of $>10 \times 10^9$/liter	40 (19.6)	26 (12.7)
Erythrocyte sedimentation rate of >30 mm/h	74 (36.3)	83 (40.7)
Serum C-reactive protein concn of >10 mg/liter	96 (47.1)	102 (50.0)
No. (%) of subjects with:		
Visible purulence at implant site	88 (43.1)	97 (47.5)
Acute inflammation on histopathology	55 (26.9)	60 (29.4)
Identical organism identified in ≥2 periprosthetic tissue specimen cultures	62 (30.4)	49 (24.0)
No. (%) of subjects in the following category:		
Prosthetic joint infection	103 (50.5)	110 (53.9)
Aseptic failure	101 (49.5)	94 (46.1)

[a]The white blood cell count was the result within 1 week prior to surgery; other laboratory findings were the results closest to the time of surgery within the 6 months preceding the surgery.

validation set were analyzed using the thresholds. The diagnostic accuracy of metagenomic sequencing was compared with that of sonicate fluid culture using McNemar's test (GraphPad, San Diego, CA). A P value of ≤0.05 was considered significant. Accuracy for predicting staphylococcal resistance was evaluated by comparison to phenotypic susceptibility.

RESULTS

Subject characteristics. A total of 408 subjects undergoing hip or knee revision or resection arthroplasties from 2011 to 2016 at Mayo Clinic, Rochester, MN, were studied. According to IDSA PJI criteria (38), among the 204 subjects in the derivation set, 103 had PJI and 101 had aseptic failure, and among the 204 subjects in the validation set, 110 had PJI and 94 had aseptic failure. The characteristics of the subjects in the two sets are shown in Table 1.

Bacterial identification in the derivation set. Using the metagenomic bacterial reports for the 204 sonicate fluid samples in the derivation set, the algorithm for positive metagenomic reports evaluates three conditions, including two thresholds for total match percentage, as follows: first, meet a unique match frequency of >15 and a relative abundance of >10%; second, bacteria belonging to the contaminant category (*Cutibacterium* spp. or *Acinetobacter* spp.) need to meet a total match percentage of >30%; and third, other bacteria need to meet a total match percentage of >3%. After following the algorithm, among the 204 sonicate fluid samples, 127 were interpreted as negative and 77 were interpreted as positive, with 68 being identified to the species level and 9 being identified to the genus level. Metagenomic analysis identified bacteria in 69.9% (72/103) of PJI subjects. Bacteria were detected in 95.0% (57/60) of culture-positive sonicate fluid samples from subjects with PJI. Among them, 44 had concordant microorganisms, 11 had partially concordant microorganisms, and 2 had discordant microorganisms detected by sonicate fluid culture (Table 2). Moreover, metagenomic analysis detected bacteria in 34.9% (15/43) of culture-negative sonicate fluid samples from subjects with PJI; 80.0% (12/15) of the culture-negative sonicate fluid samples with positive metagenomics results were from the subjects who had received antibiotics within 4 weeks preceding their resection arthroplasty. Although it missed bacteria in three culture-positive sonicate fluid samples, metagenomic analysis had a higher sensitivity than sonicate fluid culture (69.9% versus 58.3%, $P < 0.05$). Metagenomic

TABLE 2 Microorganisms detected by sonicate fluid culture and metagenomic analysis

Data set	Subject category (no. of subjects)	Microbiology results (no. of subjects)	Microorganism(s) detected (no. of subjects):	
			In sonicate fluid culture	By using metagenomic analysis of sonicate fluid
Derivation set	Prosthetic joint infection (103)	Positive sonicate fluid culture and metagenomic detection (57)		
		Concordance (44)	*Staphylococcus epidermidis* (20)	*S. epidermidis* (20)
			Staphylococcus aureus (11)	*S. aureus* (11)
			Streptococcus agalactiae (2)	*S. agalactiae* (2)
			Corynebacterium jeikeium (2)	*C. jeikeium* (2)
			Staphylococcus lugdunensis (1)	*S. lugdunensis* (1)
			Staphylococcus caprae (1)	*S. caprae* (1)
			Enterococcus faecalis (1)	*E. faecalis* (1)
			Citrobacter koseri (1)	*C. koseri* (1)
			Serratia marcescens (1)	*S. marcescens* (1)
			Granulicatella adiacens (1)	*G. adiacens* (1)
			Corynebacterium striatum (1)	*C. striatum* (1)
			Candida albicans (1)	*C. albicans* (1)
			E. faecalis + *S. epidermidis* (1)	*E. faecalis* + *S. epidermidis* (1)
		Partial concordance (11)		
		Additional organism(s) found by sonicate fluid culture or metagenomic detection (7)	*S. agalactiae* + *Peptoniphilus indolicus* (1)	*S. agalactiae* + *Peptoniphilus harei* (1)
			S. aureus + *Porphyromonas* sp. (1)	*S. aureus* (1)
			S. aureus (1)	*S. aureus* + *P. harei* (1)
			S. epidermidis (1)	*S. epidermidis* + *Finegoldia magna* (1)
			S. aureus + *S. epidermidis* (1)	*S. epidermidis* (1)
			Viridans group *Streptococcus* sp. + *Klebsiella oxytoca* + *Corynebacterium* sp. (1)	*Streptococcus mitis* (1)
			Actinomyces odontolyticus + *Peptoniphilus* sp. + *Anaerococcus vaginalis* + *S. agalactiae* + *Enterobacter aerogenes* (1)	*Actinomyces* sp. + *P. harei* + *A. vaginalis* + *S. agalactiae* + *Anaerococcus obesiensis* (1)
		Group- or genus-level identification only by sonicate fluid culture or metagenomic detection (4)	*Cutibacterium acnes* (1)	*Cutibacterium* sp. (1)
			Viridans group *Streptococcus* sp. (1)	*Streptococcus* sp. (1)
			S. epidermidis (1)	*Staphylococcus* sp. (1)
			Corynebacterium amycolatum (1)	*Corynebacterium* sp. (1)
		Discordance (2)	*Pseudomonas aeruginosa* (2)	*S. epidermidis* (2)
		Metagenomic detection and negative sonicate fluid culture (15)		*S. aureus* (3)
				S. epidermidis (2)
				Staphylococcus sp. (2)
				Streptococcus dysgalactiae (1)
				E. faecalis (1)
				Clostridium perfringens (1)
				G. adiacens (1)
				Enterobacter cloacae (1)
				Streptococcus equinus + *Streptococcus pasteurianus* (1)
				Streptococcus sp. + *Staphylococcus* sp. (1)
				Aerococcus urinae + *Peptoniphilus* sp. (1)
		No metagenomic detection with positive sonicate fluid culture (3)	*C. jeikeium* (1)	
			C. striatum (1)	
			P. aeruginosa (1)	
		No metagenomic detection with negative sonicate fluid culture (28)		
	Aseptic failure (101)	Metagenomic detection and negative sonicate fluid culture (5)		*S. aureus* (1)
				Streptococcus thermophilus (1)
				Bacteroides fragilis (1)
				Rothia sp. (1)
				Facklamia sp. (1)
		No metagenomic detection and negative sonicate fluid culture (96)		
Validation set	Prosthetic joint infection (110)	Positive sonicate fluid culture and metagenomic detection (52)		
		Concordance (39)	*S. epidermidis* (16)	*S. epidermidis* (16)
			S. aureus (5)	*S. aureus* (5)
			S. agalactiae (3)	*S. agalactiae* (3)

(Continued on next page)

TABLE 2 (Continued)

Data set	Subject category (no. of subjects)	Microbiology results (no. of subjects)	Microorganism(s) detected (no. of subjects): In sonicate fluid culture	By using metagenomic analysis of sonicate fluid
			S. lugdunensis (2)	*S. lugdunensis* (2)
			G. adiacens (2)	*G. adiacens* (2)
			Staphylococcus capitis (1)	*S. capitis* (1)
			Streptococcus salivarius (1)	*S. salivarius* (1)
			Enterococcus faecium (1)	*E. faecium* (1)
			E. faecalis (1)	*E. faecalis* (1)
			Aggregatibacter aphrophilus (1)	*A. aphrophilus* (1)
			Bacteroides fragilis (1)	*B. fragilis* (1)
			Cutibacterium avidum (1)	*C. avidum* (1)
			C. acnes (1)	*C. acnes* (1)
			Corynebacterium pyruviciproducens (1)	*C. pyruviciproducens* (1)
			C. striatum (1)	*C. striatum* (1)
			S. epidermidis + *C. amycolatum* (1)	*S. epidermidis* + *C. amycolatum* (1)
		Partial concordance (12)		
		Additional organism(s) found by sonicate fluid culture or metagenomic detection (7)	*S. epidermidis* + *E. faecalis* + *Finegoldia magna* (1)	*E. faecalis* (1)
			S. epidermidis + *Bacillus* sp. (1)	*S. epidermidis* (1)
			S. aureus (1)	*S. aureus* + *S. epidermidis* (1)
			S. aureus (1)	*S. mitis* + *S. aureus* (1)
			F. magna + *Enterobacter cloacae* (1)	*F. magna* (1)
			Enterococcus gallinarum (1)	*E. gallinarum* + *Enterococcus saccharolyticus* (1)
			S. mitis group (1)	*F. magna* + *Streptococcus oralis* (1)
		Group- or genus-level identification only by sonicate fluid culture or metagenomic detection (5)	*S. epidermidis* (2)	*Staphylococcus* sp. (2)
			Streptococcus anginosus group (1)	*Streptococcus intermedius* + *Streptococcus constellatus* (1)
			S. mitis group (1)	*Streptococcus sanguinis* (1)
			S. mitis group (1)	*Streptococcus gordonii* (1)
		Discordance (1)	*Staphylococcus pseudintermedius* + *S. epidermidis* (1)	*S. aureus* (1)
		Metagenomic detection and negative sonicate fluid culture (22)		*S. aureus* (6)
				S. dysgalactiae (3)
				S. agalactiae (3)
				S. epidermidis (1)
				Staphylococcus sp. (1)
				Pasteurella multocida (1)
				G. adiacens (1)
				Corynebacterium glutamicum (1)
				Burkholderia sp. (1)
				E. cloacae (1)
				Mycoplasma salivarium (1)
				Streptococcus sp. + *S. agalactiae* (1)
				Staphylococcus haemolyticus + *E. faecalis* (1)
		No metagenomic detection with positive sonicate fluid culture (3)	*C. acnes* (1)	
			Mycobacterium abscessus (1)	
			S. haemolyticus (1)	
		No metagenomic detection and negative sonicate fluid culture (33)		
	Aseptic failure (94)	Metagenomic detection and negative sonicate fluid culture (10)		*S. aureus* (5)
				S. mitis (1)
				S. sanguinis (1)
				Gemella sp. (1)
				S. mitis + *Streptococcus* sp. (1)
				S. sanguinis + *S. aureus* (1)
		No metagenomic detection and negative sonicate fluid culture (84)		

analysis detected bacteria in five aseptic failure subjects, which resulted in a specificity of 95.0%, compared to a specificity of 100% for sonicate fluid culture ($P = 0.07$) (Table 3). Microorganisms identified by metagenomic sequencing and sonicate fluid culture are shown in Table 2. *Staphylococcus* species were detected in 64.9% (50/77) of the positive metagenomic results, with *Staphylococcus epidermidis* (35.1%, 27/77) and *Staphylococcus aureus* (22.1%, 17/77) being the two most frequently detected organisms.

TABLE 3 Diagnostic accuracy of metagenomic analysis versus culture of sonicate fluid for diagnosis of prosthetic joint infection[a]

Date set (no. of subjects)	Method	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
Derivation set (204)	Metagenomics	69.9 (60.1, 78.6)[b]	95.0 (88.3, 98.4)	93.5 (84.9, 97.2)	75.6 (69.7, 80.7)
	Culture	58.3 (48.1, 67.9)[b]	100.0 (96.4, 100.0)	100.0 (92.5, 100.0)	70.1 (65.2, 74.7)
Validation set (204)	Metagenomics	67.3 (57.7,75.9)[b]	89.4 (81.3, 94.8)[b]	88.1 (80.2, 93.1)	70.0 (63.9, 75.5)
	Culture	50.0 (40.3, 59.7)[b]	100.0 (96.2, 100)[b]	100.0 (91.7, 100.0)	63.1 (58.6, 67.3)
Overall (408)	Metagenomics	68.5 (61.9, 74.7)[b]	92.3 (87.6, 95.6)[b]	90.7 (85.6, 94.1)	72.9 (68.7, 76.7)
	Culture	54.0 (47.1, 60.8)[b]	100.0 (98.1, 100.0)[b]	100.0 (95.9, 100.0)	66.6 (63.2, 69.7)

[a]CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b]Statistically significant difference from the other test in the same data set ($P < 0.05$).

Staphylococcal antibiotic resistance prediction in the derivation set. In the derivation set, 50 sonicate fluid samples with *Staphylococcus* species reported by metagenomic sequencing were from subjects who had staphylococci isolated from sonicate fluid, synovial fluid, or periprosthetic tissue culture and had had antibacterial susceptibility testing performed in the clinical microbiology laboratory on their culture bacteria. Among the 50 *Staphylococcus* isolates, 26 were methicillin resistant, and the *mecA* gene was detected by metagenomic analysis in 73.1% (19/26) of the associated sonicate fluid samples, with the total match percentage ranging from 47.2% to 51.8%. *mecA* was not detected in any of the 24 sonicate fluid samples in which methicillin-susceptible staphylococci were detected by culture. Twenty staphylococci were clindamycin resistant; in 70.0% (14/20) of the sonicate fluid samples in which these staphylococci were detected, an *erm* gene (*ermC* in 7 samples, *ermA* in 7 samples) was detected by metagenomic analysis. Thirty staphylococci were clindamycin susceptible, and 6 of the sonicate fluid samples in which these staphylococci were detected were positive for the *erm* gene (*ermC* in 4 samples, *ermA* in 1 sample, *ermB* in 1 sample). The total match percentage of *erm* genes ranged from 42.8% to 100%. Nine staphylococci were trimethoprim-sulfamethoxazole resistant and 41 were trimethoprim-sulfamethoxazole susceptible. In total, *dfr* genes were detected in 19 sonicate fluid samples (*dfrC* in 17 samples, *dfrC* and *dfrG* in 2 samples), with the total match percentage ranging from 58.9% to 100%. All 50 *Staphylococcus* isolates were susceptible to vancomycin; *vanA* was not detected in any of the sonicate fluid samples. Using ROC analysis to set a universal threshold for different antibiotic resistance genes for antibiotic resistance prediction, the total match percentage of >22.4% achieved the optimum sensitivity and specificity with an area under the curve (AUC) of 0.73. However, when using ROC analysis to set a threshold for each gene to achieve the optimum sensitivity and specificity, the thresholds were a total match percentage of >23.6% for *mecA* with an AUC of 0.87, >22.4% for *erm* with an AUC of 0.72, and >68.9% for *dfr* with an AUC of 0.75. The antibiotic resistance phenotype of the 50 *Staphylococcus* isolates and their respective antibiotic resistance genotypes are shown in Table 4.

Diagnostic accuracy of bacterial identification in the validation set. Using the algorithm developed with the derivation set with sequence data from 204 samples in the validation set, 120 were interpreted as negative and 84 were interpreted as positive, with 80 bacteria being identified to the species level and 4 being identified to the genus level. Metagenomic analysis identified bacteria in 67.3% (74/110) of PJI subjects. Bacteria were detected in 94.5% (52/55) of culture-positive sonicate fluid samples from subjects with PJI. Among them, 39 had concordant microorganisms, 12 had partially concordant microorganisms, and 1 had a discordant microorganism detected by sonicate fluid culture (Table 2). Furthermore, metagenomic sequencing detected bacteria in 40.0% (22/55) of culture-negative sonicate fluid samples from subjects with PJI; 77.3% (17/22) of the culture-negative samples with positive metagenomics results came from subjects who had received preoperative antibiotics in the 4 weeks preceding their surgery. Despite metagenomic analysis failing to detect bacteria in three sonicate fluid culture-positive PJI subjects, the sensitivity of metagenomic analysis was

TABLE 4 Detection of staphylococcal antibiotic resistance genes in sonicate fluid by metagenomic analysis[a]

Threshold of total match percentage [applied gene(s)]	Data set (no. of staphylococci)	Antibiotic(s)	No. of staphylococci resistant by phenotype		No. of staphylococci susceptible by phenotype		Sensitivity (% [95% CI])	Specificity (% [95% CI])
			Resistant by genotype	Susceptible by genotype	Resistant by genotype	Susceptible by genotype		
>22.4% (all genes)	Derivation set (50)	Methicillin	19	7	0	24	73.1 (52.2, 88.4)	100.0 (85.8, 100.0)
		Clindamycin	14	6	6	24	70.0 (45.7, 88.1)	80.0 (61.4, 92.3)
		TMP-SMX	7	2	12	29	77.8 (40.0, 97.2)	70.7 (54.5, 83.9)
		Vancomycin	0	0	0	50	NA	100.0 (91.1,100.0)
	Validation set (40)	Methicillin	22	5	0	13	81.5 (61.9, 93.7)	100.0 (75.3, 100.0)
		Clindamycin	9	6	2	23	60.0 (32.9, 83.7)	92.0 (74.0, 99.1)
		TMP-SMX	10	1	10	19	90.9 (58.7, 99.8)	65.5 (45.7, 82.1)
		Vancomycin	0	0	0	40	NA	100.0 (91.2, 100.0)
	Overall (90)	Methicillin	41	12	0	37	77.4 (63.8, 87.7)	100.0 (90.5, 100.0)
		Clindamycin	23	12	8	47	65.7 (47.8, 80.9)	85.5 (73.4, 93.5)
		TMP-SMX	17	3	22	48	85.0 (62.1, 96.8)	68.6 (56.4, 79.2)
		Vancomycin	0	0	0	90	NA	100.0 (95.9, 100.0)
>23.6% (*mecA*)	Derivation set (50)	Methicillin	19	7	0	24	73.1 (52.2, 88.4)	100.0 (85.8, 100.0)
		Clindamycin	14	6	6	24	70.0 (45.7,88.1)	80.0 (61.4,92.3)
>22.4% (*erm*)		TMP-SMX	7	2	10	31	77.8 (40.0,97.2)	75.6 (59.7,87.6)
		Vancomycin	0	0	0	50	NA	100.0 (91.1,100.0)
>68.9% (*dfr*)	Validation set (40)	Methicillin	22	5	0	13	81.5 (61.9, 93.7)	100.0 (75.3, 100.0)
		Clindamycin	9	6	2	23	60.0 (32.9,83.7)	92.0 (74.0,99.1)
		TMP-SMX	9	2	10	19	81.8 (48.2,97.7)	65.5 (45.7,82.1)
		Vancomycin	0	0	0	40	NA	100.0 (91.2, 100.0)
	Overall (90)	Methicillin	41	12	0	37	77.4 (63.8, 87.7)	100.0 (90.5, 100.0)
		Clindamycin	23	12	8	47	65.7 (47.8, 80.9)	85.5 (73.4, 93.5)
		TMP-SMX	16	4	20	50	80.0 (56.3, 94.3)	71.4 (59.4, 81.6)
		Vancomycin	0	0	0	90	NA	100.0 (95.9, 100.0)

[a]TMP-SMX, trimethoprim-sulfamethoxazole; CI, confidence interval; NA, not applicable.

higher than that of sonicate fluid culture (67.3% versus 50.0%, $P < 0.05$). However, metagenomic analysis found bacteria in 10 aseptic failure subjects, which resulted in a specificity of 89.4%, compared to a specificity of 100% for sonicate fluid culture ($P < 0.05$) (Table 3). The microorganisms identified by metagenomic sequencing and sonicate fluid culture are shown in Table 3. *Staphylococcus* species were identified in 54.8% (46/84) of the metagenomics-positive samples, with *S. aureus* (23.8%, 20/84) and *S. epidermidis* (22.6%, 19/84) being the two most common organisms detected. Bacterial reports and the final interpretations for the CosmosID platform, as well as for LMAT and MetaPhlAn2 analyses for individual samples, are shown in Table S1 in the supplemental material, with LMAT and MetaPhlAn2 results reproduced from reference 31 with permission from Oxford University Press. Overall, among 408 sonicate fluid samples, CosmosID metagenomics detected bacteria in 94.8% (109/115) of culture-positive sonicate fluid samples from subjects with PJI and 37.8% (37/98) of culture-negative sonicate fluid samples from subjects with PJI, with 78.4% (29/37) of the detected sonicate fluid culture-negative PJIs being from patients who had received preoperative antibiotics.

Validation of staphylococcal antibiotic resistance prediction in the validation set. In the validation set, 40 staphylococci reported by metagenomic analysis were detected by sonicate or synovial fluid or periprosthetic tissue culture, and antibacterial susceptibility testing was performed.

(i) Methicillin. Twenty-seven of the 40 staphylococci were methicillin resistant, and *mecA* was detected in 81.5% (22/27) of the sonicate fluid samples from which these staphylococci were isolated, with the total match percentage ranging from 45.8% to 51.6%. The *mecA* gene was not detected in any of the 13 sonicate fluid samples in which methicillin-susceptible staphylococci were detected.

(ii) Clindamycin. Fifteen of the 40 staphylococci were clindamycin resistant, and an *erm* gene was detected by metagenomic analysis in 60.0% (9/15) of their respective sonicate fluid samples (*ermC* in 5, *ermA* in 4). Twenty-five isolates were clindamycin susceptible, and an *erm* gene was detected in 2 of the respective sonicate fluid samples (*ermA* in 1, *erm* without a specific type in 1). The *erm* total match percentage ranged from 42.8% to 83.8%.

(iii) Trimethoprim-sulfamethoxazole. Eleven of the 40 staphylococci were trimethoprim-sulfamethoxazole resistant, and 29 were trimethoprim-sulfamethoxazole susceptible. In total, *dfr* genes were detected in 20 sonicate fluid samples (*dfrC* in 15, *dfrC* and *dfrG* in 5). The *dfr* total match percentage ranged from 40.7% to 100%.

(iv) Vancomycin. All of the 40 staphylococci were susceptible to vancomycin, and *vanA* was not detected in any of the respective sonicate fluid samples.

The antibiotic resistance phenotypes of the 40 staphylococci and the genes detected in sonicate fluid by metagenomic analysis are shown in Table 4.

DISCUSSION

In this study, we used a commercial metagenomic data analysis service to analyze metagenomic sequencing data for 408 sonicate fluid samples. We established thresholds for determining positive metagenomic results for PJI pathogens and staphylococcal antibiotic resistance genes using a derivation set and then tested them in a validation set. Metagenomic analysis demonstrated a higher sensitivity than sonicate fluid culture for the diagnosis of PJI (68.5% versus 53.5%, $P < 0.05$). In line with the findings of our previous study, metagenomic analysis in the present study detected bacteria in 94.8% (109/115) of sonicate fluid culture-positive PJIs and in 37.8% (37/98) of sonicate fluid culture-negative PJIs (similar to the 43.9% reported in the previous study [31]). Metagenomic analysis showed sensitivities ranging from 65.7% to 85.0% for the prediction of staphylococcal resistance by detecting antibiotic resistance genes.

Metagenomic sequencing is being used to identify pathogens in clinical settings. However, some obstacles of its application include the high cost of sequencing, long turnaround times, and a lack of automated informatics tools. With the development of rapid sequencing technology, costs and sequencing time may decrease. Automated bioinformatics tools are being developed to efficiently translate the overwhelming

amount of genomics data into easily understandable reports (23, 32). In our previous metagenomic study of the 408 sonicate fluid samples, we demonstrated metagenomic shotgun sequencing to be a powerful tool for detecting PJI pathogens (31). However, the turnaround time of sequencing data analysis in that study ranged from 1 to 8 h, depending on the makeup of the samples. This is longer than that for the CosmosID platform used in this study, which took 5 to 10 min from inputting raw sequencing data to final pathogen and antibacterial resistance gene reporting. The CosmosID platform abrogates the need for expert in-house microbial bioinformatics expertise.

The results of our study are broadly consistent with those of similar approaches but with some unique aspects. A recent metagenomic study of sonicate fluid by Street et al. reported that metagenomic sequencing reached a species-level sensitivity of 88% and a genus-level sensitivity of 93% compared with the results of standard aerobic and anaerobic culture of sonicate fluid (28). However, sensitivity was calculated by taking each species identified from each culture-positive sonicate sample. The authors did not review clinical information and were unable to assess accuracy for diagnosis of PJI (28). In another study, Tarabichi et al. used metagenomic sequencing targeting the 16S rRNA gene and internal transcribed spacer regions applied to synovial fluid, deep tissue, and swab specimens for diagnosing PJI and reported a sensitivity of 71.4% and a specificity of 94.6% (30). Targeted metagenomic sequencing differs from shotgun metagenomic sequencing, which was used here; for example, it does not detect viruses or bacterial genetic characteristics, such as antibiotic resistance genes. Metagenomic shotgun sequencing is largely unbiased and able to detect nucleic acid from any organism type, including bacteria, viruses, and eukaryotic microbes, as well as antibiotic resistance genes and other genetic characteristic traits (39). Notably, in this study, we evaluated only DNA and not RNA, and as such, our approach would not detect RNA viruses.

It is worth noting that the sensitivity of sonicate fluid culture was 54.0% in this study, which is lower than that of our previous study of tissue culture in blood culture bottles versus sonicate fluid culture (73.1%) (6). The reason for the discrepancy is because the sonicate fluid samples used in the present study did not include all consecutive sonicate fluid samples cultured in the clinical microbiology laboratory, with more culture-negative sonicate fluid samples being selected for the metagenomic study. In this study, metagenomics had a higher sensitivity than sonicate fluid culture (68.5% versus 54.0%, $P < 0.05$) and detected bacteria in 37.8% (37/98) of culture-negative sonicate fluid samples from subjects with PJI, most of whom (78.4%, 29/37) had received preoperative antimicrobials, rendering metagenomics superior to culture in detecting pathogens in patients on antimicrobials. However, it should be acknowledged that metagenomics may detect dead organisms.

Our study showed sensitivities of 77.4% for methicillin resistance prediction and 65.7% for clindamycin resistance prediction. Unsurprisingly, detection of antibiotic resistance using metagenomics had a lower sensitivity than assessment of cultured isolates using whole-genome sequencing. Gordon et al. (8) applied whole-genome sequencing for detecting antibiotic resistance genes from *S. aureus* isolates and obtained sensitivities of 99.0% for methicillin resistance prediction and 97.0% for clindamycin resistance prediction. Such differences in sensitivity might be explained by the fact that whole-genome sequencing of a pure isolate often secures the nearly complete coverage of entire genomes, including areas of the genomes with cassette-like properties and mobile genetic elements encoding antibiotic resistance genes, whereas metagenomic shotgun sequencing may not represent the entire genomes of every organism present and, hence, may lack read representation of genes of interest. Ruppe et al. analyzed samples for 24 patients with bone and joint infections, including 9 cases of staphylococcal infection, using metagenomic sequencing (29). They reported correct antibiotic susceptibility prediction in 94.1% of cases, based on antibiotic resistance determinant finding (29). Notably, however, they only included culture-positive samples of the same type analyzed in their analysis, which may have increased the predictive power of metagenomics for resistance prediction compared to that in our study, in which we did not exclude culture-negative sonicate fluid samples if other

specimens (i.e., periprosthetic tissue or synovial fluid) were culture positive. Of note, in this study, *mecR* and/or *mecI* was also detected in the sonicate fluids with positive detection of the *mecA* gene.

In this study, a universal threshold of a >22.4% total match percentage was established by ROC analysis for predicting staphylococcal antibiotic resistance by detection of resistance genes. CosmosID suggests using a total match percentage of ≥40% as the threshold. Since the total match percentages of all antibiotic resistance genes in this study were above 40%, using the threshold of ≥40% would yield the same results as using the threshold of >22.4%. We used *dfr* for trimethoprim-sulfamethoxazole resistance prediction instead of just trimethoprim resistance prediction because trimethoprim-sulfamethoxazole is routinely tested in the clinical microbiology laboratory; we acknowledge that the *dfr* gene confers trimethoprim resistance (8) and that we did not specifically assess for sulfamethoxazole resistance.

There are several limitations to this study. First, the diagnostic accuracy of metagenomic analysis reflects the genome sequence databases used, which are continuously being updated. However, as databases expand and include more pathogen genomes, previously unknown pathogens may be detected, which will then further improve metagenomic diagnostic accuracy. Second, in this study, *Pseudomonas aeruginosa* was missed by metagenomic data analysis but not by sonicate fluid culture. A possible reason may be MolYsis treatment, which enriches microorganisms through the lysis of host cells, and may have degraded *P. aeruginosa* DNA (31). Third, we did not evaluate viral reports from the metagenomic data analysis and may therefore have missed some viruses as potential PJI pathogens. The reason that we did not analyze for viruses in the current study is that we did not have a mechanism to establish thresholds for interpreting metagenomic viral reports. In addition, we were unable to evaluate every antibiotic resistance gene detected by metagenomic analysis because staphylococcal antibiotic phenotype testing was limited to certain antibiotics based on the institutional standard testing. Fourth, we were unable to retrieve staphylococcal isolates for further antibiotic resistance phenotype or resistance gene confirmation. Lastly, a lack of detection of an antibacterial resistance gene in some instances may be reflected by the lack of read representation of the resistance genes of interest in the sequence data. Further study of metagenomic analysis of antibiotic resistance genes, along with available isolates, for phenotype and genotype confirmation is needed. Nevertheless, to the best of our knowledge, this is the largest study predicting staphylococcal antibacterial resistance from sonicate fluid samples by metagenomic analysis.

In conclusion, a simple and easy-to-follow algorithm for interpreting metagenomic data has been established by CosmosID; this analysis has the potential to provide fast, reliable pathogen identification reports from metagenomic shotgun sequencing of pathogens in sonicate fluid samples for the diagnosis of PJI. However, strategies for metagenomic detection of antibacterial resistance genes in sonicate fluid samples for the prediction of staphylococcal antibacterial resistance need further development.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01182-18.

SUPPLEMENTAL FILE 1, XLS file, 0.5 MB.

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