Next-Generation Sequencing In Bone and Soft Tissue Infection

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Background

-Bone and soft-tissue infections (BSTIs) are challenging to diagnose and treat, regularly requiring repeat cultures, serial debridement, and multiple courses of antibiotics.

-Culture-based detection is challenged by prior antibiotic administration, fastidious organisms, and polymicrobial infections.

-Fungal and acid-fast bacilli (AFB) cultures are plagued by extensive turnaround times (TATs).

-We hypothesize that, in comparison to culture, next-generation sequencing (NGS) will provide a greater wealth of microbial data, produce comparable or faster turnaround times (TATs), and serve as an economical clinical addition.

-To the author's knowledge, this is the first study to both compare costs and TATs for NGS and all four culture modalities (aerobic, anaerobic, fungal, and acid-fast bacilli) in the context of orthopedic infections.

Methods and Demographics

-We retrospectively identified and reviewed 26 patients presenting with confirmed or suspected BSTIs requiring surgical biopsy and/or surgical irrigation and debridement from May 2023 to August 2024.

-All patients received both NGS and cultures and were analyzed regarding identified organisms, TATs, and cost. Clinical findings were provided for medical context.

-All cultures were performed at the University of Colorado Hospital. NGS was outsourced to MicroGenDX (Lubbock, Texas, USA) using the OrthoKEY Surgery protocol.

-Bone and/or tissue samples were collected at the start of the operation before application of local anesthetic. The sample was sectioned with equal partitions sent for culture and NGS.

-For microbiological analysis, data were organized into four groups, each containing a combination of positive and/or negative culture and NGS results.

-Of n=26 patients, 15/26 (57.7%) were male. Mean and median ages at the time of operation were 60.2 and 70.5, respectively. Age range at the time of operation was 21 to 80.

-Most patients, 23/26 (88.5%), had a prior documented history of site infection or surgery, and 17/26 (65.4%) required a subsequent intervention. Of this group, 9/26 (34.6%) required more than one intervention, with 1/26 (3.9%) requiring five or more interventions. Lastly, 3/26 (11.5%) required a rTKA or rTHA, and 1/26 (3.8%) required an above-knee amputation.

Tables and Figures

Table I: Anatomical Location and Suspected Etiology of Patient Infection

Patient Number	Anatomical Location	Hardware Present	Suspected Etiology
1.	L. Pelvis	N	TB/NTM Exposure
2.	L. Knee	Y	PJI
3.	R. Knee	Y	PJI
4.	R. Knee	N	Bacteremia
5.	L. Hip	Y	PJI
6.	R. Shoulder	Y	PJI
7.	L. Elbow	N	Prior Intervention
8.	L. Femur	N	Bone Cyst
9.	R. Knee	Y	PJI
10.	R. Hip	Y	PJI
11.	R. Hip	Y	Prior Intervention
12.	R. Chest	N	Prior Intervention
13.	R. Knee	N	Trauma
14.	R. Knee	N	Osteomyelitis
15.	L. Femur	Y	Prior Intervention
16.	L. Finger	N	Bacteremia
17.	L. Ankle	N	Tumor
18.	L. Knee	N	Prior Intervention
19.	R. Pelvis	N	Prior Intervention
20.	R. Ankle	N	Immunosup.
21.	L. Knee	N	Unclear
22.	L. Knee	Y	PJI
23.	R. Knee	N	Prior Intervention
24.	L. Pelvis	N	Unclear
25.	L. Knee	Y	PJI
26.	R. Knee	Y	PJI

Table II: Turnaround Times (days)

	Mean	Median	Range
Aerobic	4.8	5	3-7
Anaerobic	9.3	7	4-14
Fungal	29.9	30	29-31
AFB	54.5	57	39-63
NGS c-r*	7.0	6	4-11
NGS r-r†	4.5	5	2-7

*NGS collected-to-reported
†NGS received-to-reported

Table III: Microbiology (cultures)

	Aerobic	Anaerobic	Fungal	AFB
1.	-	-	-	-
2.	-	-	-	-
3.	Gram positive cocci; Candida glabrata complex	Rare Peptoniphilus species	Candida glabrata complex	-
4.	-	-	-	-
5.	Rare Candida albicans	-	Few candida abicans	-
6.	Rare Staphylococcus species	Rare Cutibacterium acnes	-	-
7.	-	-	-	-
8.	Rare Staphylococcus epidermidis	-	-	-
9.	-	-	-	-
10.	-	-	-	-
11.	Rare Staphylococcus aureus	-	-	-
12.	-	-	-	Mycobacterium abcessus
13.	-	-	-	Mycobacterium abcessus
14.	-	-	Rare Candida albicans	-
15.	-	-	-	-
16.	-	-	-	-
17.	Pleomorphic gram-positive rods	Cutibacterium acnes	-	-
18.	-	-	-	-
19.	-	Rare Cutibacterium acnes	-	-
20.	-	-	-	Mycobacterium haemophilum
21.	Few Corynebacterium striatum	-	Aspergillus tereus	-
22.	-	-	-	-
23.	-	-	-	-
24.	-	-	-	-
25.	Rare Staphylococcus lugdunensis	Few Finegoldia magna	-	Mycobacterium abscessus
26.	-	-	-	-

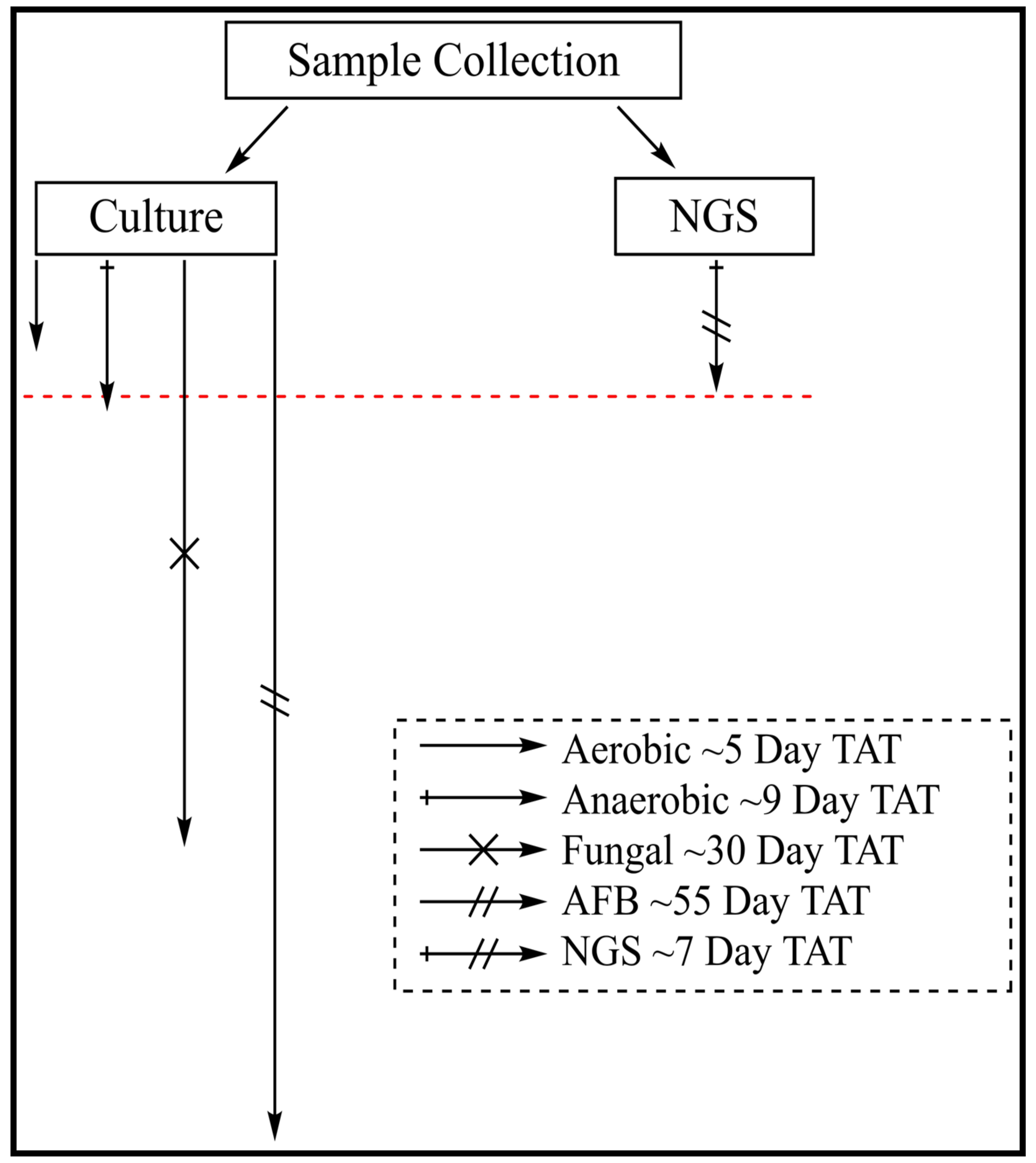

Figure 1:Visual comparison of culture and next-generation sequencing turnaround times, highlighting next-generation sequencing's timeliness in bypassing fungal and acid-fast bacilli wait times.

Table IV: Microbiology (next-generation sequencing and resistance genes)

	Next-generation sequencing	Resistance gene
1.	-	-
2.	Streptococcus oralis 97%; Granulicatella adiacens 2%	tetM
3.	Peptoniphilus vaginalis 38%; Peptoniphilus harei 18%; Staphylococcus epidermidis 8%; Anaerococcus vaginalis 6%	tetM
4.	Escherichia coli 86%	-
5.	-	-
6.	Staphylococcus epidermidis 99%	-
7.	Staphylococcus aureus 76%; Corynebacterium tuberculostearicum 21%	-
8.	-	-
9.	-	-
10.	-	-
11.	Staphylococcus aureus 100%	-
12.	Kocuria rhizophila 92%; Dermacoccus nishinomiyaensis 7%	-
13.	-	-
14.	Candida albicans 100%	-
15.	-	-
16.	Mycobacterium haemophilum 72%	-
17.	-	-
18.	-	-
19.	Arcanobacterium haemolyticum 27%; Streptococcus anginosus 24%; Finegoldia magna 16%; Cutibacterium acnes 16%; Veillonella parvula 6%; Veillonella dispar 6%; Winkia neuii 2%	-
20.	-	-
21.	Corynebacterium striatum 98%	ant2-Ia
22.	-	-
23.	-	-
24.	Pseudomonas aeruginosa 100%	-
25.	Staphylococcus lugdunensis 60%; Finegoldia magna 39%	mecA; tetM
26.	Staphylococcus epidermidis 70%	-

tetM: tetracycline resistance gene M. ant2-Ia: aminoglycoside-2″-O-nucleotidyltransferase. mecA: methicillin resistance gene A

Results

-Mean (x̄), median (x̃), and range (r) of TATs in days were as follows: aerobic (x̄ = 4.8; x̃ = 5; r = 3-7), anaerobic (x̄ = 9.28; x̃ = 7; r = 4-14), fungal (x̄ = 29.9; x̃ = 30; r = 29-31), AFB (x̄ = 54.5; x̃ = 57; r = 39-63), NGS collected-to-reported (x̄ = 6.96; x̃ = 6; r = 4-11), and NGS received-to-reported (x̄ = 4.52; x̃ = 5; r = 2-7).

-5/26 (19.2%) received negative NGS results and positive cultures, 6/26 (23.1%) received positive NGS results and negative cultures, 7/26 (26.9%) received negative NGS results and cultures, and 8/26 (30.8%) received positive NGS results and cultures.

-Within the last group, 1/8 (12.5%) had no agreement, 2/8 (25%) had full agreement, and 5/8 (62.5%) had partial agreement.

-Sending for NGS was 42.5% cheaper than ordering cultures at our institution ($433 vs $249).

-When an antibiotic susceptibility test (AST) is added, NGS becomes 56.8% cheaper ($576 vs $249).

Conclusion

-We recommend NGS as an informative, timely, and economical tool for use alongside culture-based detection in suspected or confirmed BSTIs.

-NGS may improve patient outcomes by bypassing fungal and AFB wait times and by providing identification when cultures are negative.

-NGS highlights rare and difficult-to-culture organisms in polymicrobial infections, the identities of which may be important in the setting of trauma or latent and recurring BSTIs.

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Disclosures

-The authors have no relevant financial disclosures and industry support was not provided for this study.