

Validating the use of Next-Generation Sequencing in Open/Ballistic Fracture Management

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INTRODUCTION:

Fracture site infection following open and/or ballistic fractures present a significant challenge for surgeons and patients. Despite institutional protocols and advancements in antibiotic therapy, up to 27% of these fractures will go on to develop infection. Inability to accurately identify the infective organism and antibiotic resistance play a major role in the disease process. This is in part due to limitations of standard microbiological culture leading to low sensitivity, such as, inability to detect poly-microbial infections, fastidious bacteria, or bacteria within biofilms. Advances in precision medicine have supported the use of metagenomic analysis with the stated advantage of massive parallel full genome sequencing termed "next generation sequencing" (NGS). In this study we empower the use of NGS to detect bacterial contamination of operative open and ballistic fractures. We compared the results of NGS to standard microbiological culture.

METHODS:

Following IRB approval, adult (>18 years old) patients who presented with an open or ballistic fracture at our level one trauma center were consecutively enrolled. Exclusion criteria included fractures not of the long bones or pelvis, prior surgery/hardware, and patients with known, chronic infections. Preoperatively, fractures underwent bedside irrigation with a betadine and saline solution. Intraoperatively, tissue specimens were collected using sterile instruments in accordance with standard operating procedure for procuring tissue at the beginning of the surgical case. Soft tissue and bone were collected when possible. Next-generation sequencing analysis was conducted in parallel with standard broth-based culture on both tissue specimens and intramedullary canal swabs. Standard broth-based culture included aerobic, anaerobic, fungal, and acid-fast bacterium (AFB) isolates. Retrospective chart review was performed to obtain demographic and social data for each enrolled patient.

RESULTS:

Forty samples were collected from 39 patients (1 patient had two separate injuries). 34 samples (85%) from male patients. The average age at time of injury was 43 years old (+/- 18 years). Average time to surgery was 1.17 days (+/-1.30 days). 27 samples were from open fractures while 13 samples were from ballistic fractures. 34 samples came from lower extremity bones, while 6 came from upper extremity bones. Tibial fractures encompassed the majority of fractures (22; 55%), followed by femurs (11; 27.5%), then humerus (4; 10%), forearm (2; 5%), and talus (1; 2.5%). NGS was positive in 15 samples (37.5%) while culture was positive in 9 samples (22.5%). Of the 15 positive NGS samples, 8 (53.3%) of those had a negative culture (NGS positive, culture negative), compared to only 2 (22.2%) positive culture samples that had a negative NGS (culture positive, NGS negative; table 1). In each case where both NGS and culture were both positive, the NGS assay was able to detect the same organism isolated by the culture assay. The ability to detect bacteria by NGS or culture between open fractures compared to ballistic fractures was similar (37.0% and 38.5% respectively for NGS; 22.2% and 23.1% for culture).

DISCUSSION AND CONCLUSION:

In this study, we demonstrated that NGS is a feasible tool for detecting bacterial DNA contamination in open and ballistic injuries, with a higher detection rate than traditional broth culture. In cases where both culture and NGS were positive, the NGS was able to detect the same organism isolated in culture. Additionally, NGS was more likely to detect polymicrobial contamination compared to traditional culture. Despite these positive findings, further research is necessary to clarify the clinical significance of NGS findings and their potential role in guiding treatment protocol.

Total Samples (n)	40
Average age (years)	43 (+/-18)
Sex	34 (85%) male
Time to surgery (days)	1.17 (+/- 1.30)
Open Fractures	27 (67.5%)
Ballistic	13 (32.5%)
Total NGS Positive Samples	15 (37.5%)
NGS Positive, Culture Negative	8 (53.3%)
Total Culture Positive Samples	9 (22.5%)
Culture Positive, NGS Negative	2 (22.2%)
Anatomical Location	
Tibia	22 (55%)
Femur	11 (27.5%)
Humerus	4 (10%)
Forearm	2 (5%)
Talus	1 (2.5%)

Table 1 – Patient and specimen demographic data.

Sample Identifier	NGS Results	Culture Results
1	<i>Corynebacterium tuberculostearicum</i> , <i>Corynebacterium afermentans</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus hominis</i> , <i>Staphylococcus haemolyticus</i> , <i>Staphylococcus cohnii</i> , <i>Staphylococcus aureus</i>	<i>Staphylococcus Hominis</i>
2	<i>Corynebacterium species</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus warneri</i> , <i>Staphylococcus hominis</i> , <i>Staphylococcus lugdunensis</i>	negative
3	<i>Acinetobacter junii</i>	negative
4	negative	<i>Staphylococcus epidermidis</i>
5	<i>Corynebacterium tuberculostearicum</i> , <i>Corynebacterium thomsonii</i> , <i>Cultobacterium ivolitum</i> , <i>Corynebacterium genitalium</i> , <i>Cultobacterium granulatum</i> , <i>Anaerocoocus octavus</i>	<i>Staphylococcus capitis</i> , <i>Cultobacterium (Propionibacterium) granulatum</i>
6	<i>Bacillus species</i>	negative
7	<i>Bacillus cereus</i> , <i>Microbacterium oxydans</i> , <i>Purpureocillium lilacinum</i> , <i>Fusarium oxysporum</i>	negative
8	<i>Acinetobacterwoffli</i> , <i>Acinetobacter schindleri</i>	<i>Micrococcus species</i>
9	<i>Corynebacterium tuberculostearicum</i> , <i>Staphylococcus haemolyticus</i> , <i>Staphylococcus hominis</i> , <i>Corynebacterium afermentans</i>	negative
10	<i>Acinetobacter junii</i>	
11	<i>Staphylococcus caprae</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus capitis</i>	<i>Staphylococcus capitis</i>
12	<i>Pseudomonas stutzeri</i>	<i>Pseudomonas stutzeri</i>
13	<i>Cultobacterium aeneum</i> , <i>Staphylococcus hominis</i> , <i>Staphylococcus epidermidis</i> , <i>Acinetobacter junii</i> , <i>Massilia aurea</i> , <i>Sphingomonas hankookensis</i> , <i>Brevundimonas variabilis</i> , <i>Acinetobacter towneri</i> , <i>Corynebacterium tuberculostearicum</i> , <i>Streptococcus equinus</i>	negative
14	<i>Staphylococcus hominis</i> , <i>Nosocomiocoocus massiliensis</i> , <i>Corynebacterium sp.</i> , <i>Staphylococcus cohnii</i> , <i>Staphylococcus epidermidis</i> , <i>Ascochyta hordei</i>	negative
15	negative	<i>Staphylococcus hominis</i>
16	<i>Pseudomonas korzenisii</i>	<i>Bacillus species</i>
17	<i>Pseudomonas korzenisii</i>	<i>Bacillus species</i>

Table 2: Organism(s) identified in samples with positive NGS and/or culture results.