http://dx.doi.org/10.2106/JBJS.21.00785

May 5, 2022

Next-Generation DNA Sequencing is a valuable tool for detecting bacterial presence

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Infection is one of the most dreaded complications after orthopaedic procedures, especially in the trauma setting (1). It can cause delayed healing and even lead to permanent functional loss or worse, amputation of the affected extremity. Unfortunately, diagnosing surgical site infection is a challenging process, particularly when the infecting organism is slow growing (2). There is no gold standard, and the common methods of infection detection are far from perfect. Numerous studies have shown that traditional cultures fail to isolate the pathogens in 20-42% of the cases (3–5).

Next-generation sequencing (NGS), 16S rRNA gene bacterial profiling, has shown promising results in detecting bacterial signal via identifying the presence of bacterial DNA (6).

The American Association of Microbiology has mentioned that NGS has the potential of revolutionizing the clinical microbiology laboratory by “replacing current time-consuming and labor-intensive culture techniques with a single, all-inclusive diagnostic test.” (7)

Natoli et al. (8) investigated the application of NGS in their prospective study of patients who underwent orthopaedic trauma procedures who were thought to be “clean” and compared it to that of culture. Authors had three groups; 1. patients undergoing surgical treatment for acute closed fractures, 2. patients undergoing implant removal at the site of a healed fracture without infection, and 3. patients undergoing a first procedure for the treatment of a fracture nonunion who might or might not have subclinical infection. They found that in group one, who is presumed to have no bacteria, cultures were positive in 2.3% (95% CI, 0.4% to 12%) of the patients vs. 33% (95% CI, 21% to 48%) for NGS. Similar theme was observed in the other study groups. Based on this data authors concluded that “NGS should not currently substitute for or complement conventional culture in orthopaedic trauma patients with low suspicion of infection.” Nevertheless, this should’ve not been a surprising finding. As mentioned earlier we already knew that NGS is more sensitive in detecting bacterial presence than cultures. Group 1 should’ve not been presumed to have no bacteria as the samples were obtained from the fracture hematoma. The first stage of fracture healing is inflammation which, recruits inflammatory cells and increases permeability of the capillary system causing a hematoma to form

around the fracture site (9).

Numerous pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-?), interleukins (IL-1, IL-6, IL-11, IL-23), and bone morphogenetic proteins (BMPs) are released as a result of injury to bone (10,11). These cytokines act to stimulate essential cellular biology at the site, attracting macrophages, monocytes, and lymphocytes (12). Moreover, human body constantly experiences asymptomatic bacteremia (13,14). These bacteria, which are mostly nonpathogenic (as also stated by the authors) can easily make their way into the fracture hematoma. Therefore, this should've not been a surprise to detect bacterial presence in the fracture hematoma. Traditional cultures simply failed to detect them. Authors also indicated that "Cases with elevated inflammatory markers, previous open fracture, and/or previous treatment for infection were not excluded.", which on its own can increase the risk of bacterial presence.

As for the other two groups of the study there is a significant amount of literature showing that retained hardware can harbor bacteria especially in a biofilm form that is harder to be detected by the traditional cultures (6,17) Poor agreement between cultures and NGS is expected and lack of positive culture results does not translate to absence of bacteria.

Unnecessary testing is a major problem that can mislead diagnosis and has a huge impact on the healthcare system (15,16). The authors concluded that NGS should not substitute or complement culture in orthopaedic trauma patients, and we believe that this is not supported by the findings of this paper. Neither culture nor NGS should be used in a closed fracture that is undergoing surgical treatment or patients with low likelihood of infection. This practice does not improve the care of these patients and in fact can cause confusion and imposes additional cost. The fact that cultures failed to detect presence of bacteria in these patients does not mean that there was no bacterial presence. And positive NGS results that indicated bacterial presence does not mean that there would be increased risk for infection. Therefore, we recommend against performing either test without any signs of infection and a host response. Authors appear to agree with the aforementioned statement and in their discussion mentioned "A secondary purpose of the current study was to assess the relationship between culture or NGS positivity and the development of postoperative surgical site infection." They found that there is no relationship between postoperative infection and routine culture results and NGS, therefore, routine testing is not worthwhile.

NGS is a sensitive and specific test for detecting bacterial presence and has been repeatedly shown to have a better performance than traditional cultures. However, like any other tool it should be used in the appropriate setting.

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Conflict of Interest: None Declared