Use of a Scattered Light Integrating Collector for Point of Care Testing to detect Bacteria in Fluid Samples from Prosthetic Joint Infections of the Hip

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INTRODUCTION:

In the United Kingdom, 72,000 total hip replacements are undertaken annually, with around 0.5-1% becoming infected. This serious complication has a significant associated mortality, a profound psychological burden on patients and surgeons, and a non-negligible economic cost.

Early targeted antibiotic therapy impacts patient outcomes, and implementation of the sepsis-6 protocol (which calls for initiation of antibiotic therapy within 1 hour) is recommended in the BOAST guidelines for prosthetic joint infection with evidence of sepsis. Early treatment with targeted narrow spectrum antibiotics is limited by the available methods of detecting infection and antimicrobial sensitivities. This leads to a period of empirical treatment with broad spectrum antibiotics, which represents poor antimicrobial stewardship, allows resistance to develop, and may see the clinical deterioration of the patient if the organism is not susceptible to the chosen antimicrobial.

We propose a novel diagnostic technology, the Scattered Light Integrating Collector (SLIC) which can facilitate the early and accurate identification of organisms and sensitivities,. SLIC is a simple, low-cost, label-free method, that lowers the limit of bacterial detection, allowing the study of bacterial growth dynamics in real time. The scatter pattern of a red laser beam is utilised to detect and quantify organisms in suspension, with a maximal sensitivity of 100 colony forming units (CFU)/mL. This allows the presence of infection to be detected, and antimicrobial sensitivity testing (AST) to be completed rapidly.

Having established the efficacy of SLIC in gram negative blood cultures and urine samples, we now investigate its utility in diagnosing and establishing sensitivities in the context of organisms typical for prosthetic joint infection (PJI).

METHODS:

Six organisms common in PJI (based on literature search) were included in our study: ATCC strains S. aureus (27660), E. coli (25922), E. faecalis (29212), and patient-derived MRSA, S. epidermidis, and S. pyogenes. All were laboratory stocks, stored at -70 degrees, and subcultured in Muller Hinton Cation Broth overnight prior to use.

Six antibiotics typically used in PJIs were selected for testing: vancomycin, gentamicin, flucloxacillin, rifampicin, cefuroxime, and cotrimoxazole.

We assessed the degree of concordance between SLIC and the current standard of care for AST – 16 hour broth microdilution (BMD).

Each organism was tested against each antibiotic, resulting in 36 test combinations, all performed in triplicate technical repeats. The minimum inhibitory concentration (MIC) for each antibiotic against each organism was derived from the EUCAST database where available, otherwise was experimentally derived via BMD. Each antibiotic was tested at 5 concentrations: MIC, and 2 doubling dilutions above and below. A positive control condition with no antibiotic was included.

Time to positivity (TTP) for infection was defined as the time at which the lower bound of the positive control trace +/- standard error of the mean crossed and remained permanently above the X axis.

An MIC90 (90% growth inhibition relative to positive control) was derived directly from BMD in each test combination, and was used as a cutoff to determine susceptibility. For SLIC, the Area Under the Curve (AUC) was derived for each growth trace. The AUC90 (AUC < 90% that of the positive control) and AUC 50 (AUC < 50% that of the positive control) were investigated as cutoffs for susceptibility, and were tested against the MIC90 at 60 and 120 minutes for essential agreement (EA) and categorical agreement (CA). Rates of Major Error (ME – BMD indicates susceptibility, SLIC indicates resistance) and Very Major Error (VME – BMD indicates resistance, SLIC indicates susceptibility) were calculated. Predictive values for resistance were derived.

Finally, a previous pilot case evaluation was carried out at Bonn University Hospital. With patient consent, intraoperative samples were obtained at the time of revision surgery for a total hip replacement known to be infected with Propionibacterium. These were run through SLIC and TTP and time to AST were obtained.

RESULTS:

TTP for all organisms was <30 minutes. AUC50 at 60 minutes gave essential agreement of 75% and categorical agreement of 81% against BMD, with a positive predictive value (PPV) of 86% for resistance. AUC50 performed less well than AUC90 at 120 minutes (67% categorical and essential agreement vs 72% categorical and essential agreement respectively), due to a higher rate of VME (22% for AUC50 vs 8.3% for AUC90), though AUC50 PPV for resistance remained high at 94%.

In the previous pilot study, SLIC was able to detect the presence of bacterial infection in under 30 minutes, with AST also completed in under 30 minutes. On the basis of these results and the previous pilot study, we infer that the patient could have been started on an antibiotic sensitive for their specific prosthetic joint infection within the time taken to complete their revision surgery.

DISCUSSION AND CONCLUSION:

We demonstrate that in organisms typical for PJI, SLIC detects infection rapidly, shows a high PPV for the presence of antimicrobial resistance, and has strong concordance with BMD in defining AST, at 60 and 120 minutes. This timescale compares favourably to the current laboratory standard (culture and colorimetry), which typically returns results in 48h or longer. SLIC also obviates the need for a priori knowledge pertaining to the likely organism, as is often required by rapid molecular methods.

Having successfully defined the utility of SLIC in laboratory cultivated organisms, we plan to expand the use of SLIC to a clinical setting, defining a robust standard operating procedure for growth detection and AST in patient synovial fluid samples, tissue samples from infected joints, and sonication fluid from explanted metalwork, thus establishing SLIC as a rapid and accurate method for informing early antimicrobial therapy in PJIs.