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Matrix-assisted laser desorption ionization time of flight mass spectrometry and diagnostic testing for prosthetic joint infection in the clinical microbiology laboratory

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ABSTRACT

Identification of pathogen(s) associated with prosthetic joint infection (PJI) is critical for patient management. Historically, many laboratories have not routinely identified organisms such as coagulase-negative staphylococci to the species level. The advent of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) has enhanced clinical laboratory capacity for accurate species-level identification. The aim of this study was to describe the species-level identification of microorganisms isolated from periprosthetic tissue and fluid specimens using MALDI-TOF MS alongside other rapid identification tests in a clinical microbiology laboratory. Results of rapid identification of bacteria isolated from periprosthetic joint fluid and/or tissue specimens were correlated with clinical findings at Mayo Clinic, Rochester, Minnesota, between May 2012 and May 2013. There were 178 PJI and 82 aseptic failure (AF) cases analyzed, yielding 770 organisms (median, 3/subject; range, 1–19/subject). MALDI-TOF MS was employed for the identification of 455 organisms (59%) in 197 subjects (123 PJIs and 74 AFs), with 89% identified to the species level using this technique. Gram-positive bacteria accounted for 68% and 93% of isolates in PJI and AF, respectively. However, the profile of species associated with infection compared to specimen contamination differed. *Staphylococcus aureus* and *Staphylococcus caprae* were always associated with infection, *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* were equally likely to be a pathogen or a contaminant, whereas the other coagulase-negative staphylococci were more frequently contaminants. Most streptococcal and *Corynebacterium* isolates were pathogens. The likelihood that an organism was a pathogen or contaminant differed with the prosthetic joint location, particularly in the case of *Propionibacterium acnes*. MALDI-TOF MS is a valuable tool for the identification of bacteria isolated from patients with prosthetic joints, providing species-level identification that may inform culture interpretation of pathogens versus contaminants.

1. Introduction

Microbiological identification of pathogens associated with prosthetic joint infection (PJI) allows for institution of appropriate management strategies for infection. A number of challenges exist in the diagnosis of PJI, in particular, differentiating infection from specimen contamination. The most common causative agents of PJI are conversely organisms frequently associated with specimen contamination (Harris et al., 2010; Osmon et al., 2013; Parvizi et al., 2011; Zimmerli et al., 2004). Coagulase-negative *Staphylococcus* species (CoNS) are the prototypical example; CoNS are commensal skin organisms that are frequent laboratory contaminants but also account for 25–50% of PJIs (Harris et al., 2010; Osmon et al., 2013; Steckelberg and Osmon, 2000; Zimmerli et al., 2004). Established diagnostic tests exist for rapid organism identification of a limited number of organisms. However, until now, there has not been a rapid way to identify most bacteria and fungi isolated from the site of a prosthetic joint, and conventional (nonrapid) microbiological techniques have had a limited capacity to identify most isolated organisms to the species level (Harris et al., 2010). Moreover, traditional methods for identification of most isolated organisms to the species level are associated with significant cost. For these reasons, organisms such as the CoNS and *Corynebacterium* species have not historically been identified to the species level on a routine basis. Doing so would have required sophisticated and expensive biochemical and/or molecular testing and would not have been rapid. Although limited identification of these organisms has been accepted in clinical practice, it is possible that clinical differentiation between contaminated specimens and infection might be aided by species-level identification of these organisms.

With the advent of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), rapid and accurate identification of bacteria to the species level is possible in routine clinical

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practice (Borens et al., 2012; Harris et al., 2010). This, in turn, has the potential to inform our understanding of the clinical significance of the isolation of specific species from the site of a prosthetic joint. Beginning in 2011, MALDI-TOF MS was iteratively introduced into our routine clinical practice, such that since 2012, it has been used to identify most bacteria isolated from the site of a prosthetic joint. It is used alongside conventional rapid diagnostic tests such as catalase, coagulase, indole, and oxidase testing for the identification of microorganisms from sterile site specimens. Herein, we addressed the clinical significance of individual species of bacteria and fungi isolated from the site of a prosthetic joint using current-day, rapid identification tests with a particular focus on the role of MALDI-TOF MS. We also explored the question as to how many culture-positive specimens should be tested with MALDI-TOF MS in the case of multiple culture-positive specimens.

2. Patients and methods

2.1. Study design

This retrospective cohort study examined the impact of the addition of MALDI-TOF MS to a battery of conventional diagnostic microbiological testing for rapid identification of bacteria or fungi isolated from periprosthetic tissue and fluid specimens. Patients were identified from a review of the computerized records of the Division of Clinical Microbiology in which ≥1 microorganism(s) had been isolated from periprosthetic tissue or fluid samples over a 12-month period (May 2012–May 2013). A comprehensive chart review was performed by a single researcher (TP). The study design was reviewed and approved by the institutional review board (IRBe 13-005295).

2.2. Definitions

PJI was defined if patients met criteria of either the Infectious Diseases Society of America (IDSA) or the Musculoskeletal Infection Society (MSIS) definition of PJI (Table 1) (Osmon et al., 2013; Parvizi et al., 2011). "Aseptic failure" (AF) was defined if patients did not meet either criteria for PJI. A specific microorganism was considered a "pathogen" if isolated from ≥2 separate periprosthetic tissue or fluid specimens (Trampuz et al., 2007; Zimmerli et al., 2004). Culture results were defined as "indeterminate" if a specific microorganism was isolated from a single culture only in a patient otherwise meeting criteria for PJI. Microorganisms were classified as "contaminants" if isolated from a single culture in a patient with AF (Atkins et al., 1998).

Table 1
IDSA and MSIS criteria for prosthetic joint infection.

IDSA definition	MSIS definition
1. Sinus tract communicating with the prosthesis; or 2. Acute inflammation present on histopathologic examination of periprosthetic tissue as defined by the attending pathologist; or 3. The presence of purulence surrounding the prosthesis; or 4. 2 or more intraoperative cultures or combination of preoperative aspiration and intraoperative cultures yield the same indistinguishable organism (Osmon et al., 2013).	1. Sinus tract communicating with the prosthesis; or 2. A pathogen is isolated by culture from at least 2 separate tissue or fluid samples obtained from the affected prosthetic joint; or 3. 4 of the following 6 criteria exist: a. Elevated erythrocyte sedimentation rate and C-reactive protein b. Elevated synovial leukocyte count c. Elevated synovial neutrophil percentage d. Presence of purulence in the affected joint e. Isolation of a microorganism in one culture of periprosthetic tissue or fluid, or f. Acute inflammation present on histopathologic examination of periprosthetic tissue as defined by the attending pathologist[a] (Parvizi et al., 2011).

[a] Modified, in keeping with current practice at our center, from the MSIS criteria: greater than 5 neutrophils per high-power field in 5 high-power fields observed from histologic analysis of periprosthetic tissue at magnification ×400.

2.3. Periprosthetic tissue and fluid, synovial fluid, and sonicate culture methods

Fluids and tissues were collected into anaerobic fluid vials (20-mL serum stopper vials with 1.3 mL prereduced peptone yeast extract broth, 0.5 g/L cysteine hydrochloride, and 1 mg/L resazurin indicator) and tissue vials (sterile 30-mL screw-top vials filled with CO_2), respectively. Tissues were homogenized using a Seward Stomacher 80 Biomaster (Seward, Port St. Lucie, FL, USA) in 3 mL of brain–heart infusion broth for 1 min and inoculated as follows: 0.1 mL was inoculated onto sheep blood and chocolate agar and incubated aerobically at 35°C in 5% CO_2 for 4 days; 0.1 mL was inoculated onto anaerobic sheep blood agar and incubated anaerobically for 14 days; and 1 mL was inoculated into enriched thioglycollate broth (BD Diagnostic Systems, Sparks, MD, USA), incubated at 35°C for 14 days, and subcultured if cloudy. For aerobic culture of synovial fluids, volumes 1 mL or greater were inoculated into a BACTEC Peds Plus/F blood culture bottle (Becton Dickinson, Oxford, UK) and incubated for 5 days; bottles were subcultured if the instrument flagged as positive. Smaller synovial fluid volumes were inoculated onto sheep blood and chocolate agar and incubated aerobically as described above. In addition, all synovial fluid aspirates were inoculated onto anaerobic sheep blood agar and into enriched thioglycollate broth and incubated as described above. At the treating surgeon's discretion and in keeping with current consensus recommendations, removed implants underwent vortexing and sonication in Ringer solution followed by centrifugation. 0.1 mL of the concentrated sonicate fluid was inoculated onto aerobic and anaerobic sheep blood agar plates, which were incubated aerobically at 35°C in 5% CO_2 for 4 days and anaerobically for 14 days, respectively (Piper et al., 2009; Trampuz et al., 2007; Zmistowski et al., 2014).

2.4. Microorganism identification

During the course of this pragmatic, clinical laboratory-based study, MALDI-TOF MS was gradually introduced into our routine clinical practice after completion of internal laboratory validation studies for different groups of microorganisms encountered. MALDI-TOF MS was not exclusively used for organism identification but was instead incorporated into the laboratory workflow, performed in conjunction with other rapid biochemical tests. MALDI-TOF MS was performed on all isolated CoNS, *Enterococcus* species, *Corynebacterium* species, and selected *Streptococcus* species and Gram-negative bacteria using the Bruker Biotyper (Bruker Daltonics, Billerica, MA, USA), as previously described (Theel et al., 2012). Library version 4110 was used from April 2012 to September 2012, and library version 4613 was used for the remainder of the study period. The manufacturer's recommended cutoffs were used to determine genus and species levels of identification, with the exception of *Corynebacterium* species and viridans group streptococci, where a lowered species-level cutoff of ≥1.700 was applied (Alatoom et al., 2012). The species with the highest score was considered the species of record with a minimum difference of 10% between the top score and next closest species' or genera's score required (Saffert et al., 2011). In addition to MALDI-TOF MS, other rapid biochemical tests performed included rapid coagulase testing to identify *Staphylococcus aureus*; rapid oxidase testing to identify *Pseudomonas aeruginosa*; rapid indole, rapid PYR, rapid oxidase, motility, and lysine testing to identify *Escherichia coli* and differentiate it from *Shigella* species; rapid catalase and rapid indole testing to identify most *Propionibacterium acnes*; rapid desoxycholate or optochin testing to differentiate *Streptococcus pneumoniae* from *Streptococcus mitis* species group; and Lancefield typing to identify β-hemolytic *Streptococcus* species. 16S rRNA gene PCR/sequencing was performed if MALDI-TOF MS and/or biochemical testing