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Original Article

Use of MALDI-TOF mass spectrometry after liquid enrichment (BD Bactec™) for rapid diagnosis of bone and joint infections

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Abstract

Advantages of MALDI-TOF MS (MS) were evaluated for diagnosis of bone and joint infections after enrichment of synovial fluid (SF) or crushed osteoarticular samples (CSs). MS was performed after enrichment of SF or crushed osteoarticular samples CS (n = 108) in both aerobic and anaerobic vials. Extraction was performed on 113 vials (SF: n = 47; CS: n = 66), using the Sepsityper® kit prior identification by MS. The performances of MS, score and reproducibility results on bacterial colonies from blood agar and on pellets after enrichment in vials, were compared. MS analysis of the vial resulted in correct identification of bacteria at a species and genus level (80.5% and 92% of cases, respectively). The reproducibility was superior for aerobic Gram-positive bacteria (*Staphylococci* and Gram-positive bacilli: 100% colonies), as compared to aerobic Gram-negative bacilli (89.7%), anaerobes (83.3%) and *Streptococcus/Enterococcus* (58.8%). MS performance was significantly better for staphylococci than for streptococci on all identification parameters. For polymicrobial cultures, identification (score>1.5) of two species by MS was acceptable in 92.8% of cases. Use of MS on enrichment pellets of bone samples is an accurate, rapid and robust method for bacterial identification of clinical isolates from osteoarticular infections, except for streptococci, whose identification to species level remains difficult.
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Keywords: MALDI-TOF mass spectrometry; Osteoarticular infection; Sepsityper® kit; Time of detection; Beadmill processing; Polymicrobial samples

1. Introduction

Direct examination is an unreliable method for the diagnosis of bone infections [1], with a sensitivity threshold assessed at an inoculum of approximately 10^4 UFC/mL. Achieving an enrichment step in a liquid medium with prolonged incubation of at least 14 days is essential [2] for correct diagnosis. This time is required to observe the growth of "small colony variants" or fastidious bacteria and to dilute any antibiotic potentially present in the synovial fluid (SF) or crushed bone samples (CSs). A biopsy beadmill processing step [3,4] or a step of sonication [5] on prosthetic samples provides improvement of culture performances. This is particularly true in the case of bacterial biofilms [6], chronic or complicated infections associated with prosthetic material.

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Infections on osteosynthesis material may be polymicrobial (10−15%) [7], and diagnosis of these infections remains difficult and often fails to identify all these bacterial species.

Universal gene amplification techniques (eg. 16SrDNA, *sodA*) are a diagnostic option, particularly in case of prior antibiotic treatment, but the time consumed (due to the necessary secondary sequencing of the amplified product), the cost of this test and its low sensitivity are major disadvantages to its use [4,8]. Specific polymerase chain reactions (PCRs) (*Borrelia, Kingella kingae, Tropheryma whipplei,* etc.) are more sensitive and specific tests, but the procedure requires targeting a single gene with a known sequence. This is a limit to its use in the context of bone and joint infections, where the pathogen is often unknown; accurate diagnosis may require laboratories to perform several specific PCRs.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry, or MALDI-TOF MS (MS), is frequently used for identification of a single colony (isolated on agar media) from clinical and environmental samples [9−11]. The MS system provides rapid and high-confidence identification of bacteria, yeasts and fungi, based on proteomic fingerprinting using high-throughput MALDI-TOF mass spectrometry. Its use has recently been extended to clinical diagnosis, either directly from positive blood culture vials [12] or from samples such as urine [13]. Research suggests that this technique is relevant for microorganism identification, with functionality comparable to routine methods used in the clinical microbiology laboratory [14]. In the case of blood culture vials, bacterial identification by MS directly on the vial pellets optimizes the rendering time result with a time-saving of 1−24 h over conventional methods depending on the extraction technique [15,16]. Results quickly available contribute to reducing morbidity [17] and mortality in addition to lower cost of treatment and length of hospital stay.

This study evaluated the usefulness of MS for rapid diagnosis of bone and joint infections. Synovial fluid (SF) or crushed osteoarticular samples (CSs) were enriched in aerobic and anaerobic blood vials before harvesting bacteria (from positive vial cultures), which were then rapidly identified by MALDI-TOF. To assess the performance of MS, score and reproducibility results on bacterial colonies, directly seeded on blood agar from the sample and on pellets after enrichment in blood vials, were compared. Additionally, we defined the detection rate of culture for SF and CS by bacterial species after enrichment in aerobic and anaerobic blood vials.

2. Material and methods

2.1. Samples − scheme of the study

This was a prospective single-center study conducted at the University Hospital of Rennes (Reference Centre for Complex Osteoarticular Infections for the West of France) from January to October 2013. Osteoarticular samples (OASs) were collected and analyzed at the Laboratory of Bacteriology within 2 h of receipt after possible storage at room temperature. Synovial fluids (SFs) were collected in a sterile tube (Falcon®) and bone samples in a sterile jar (30 mL, HDPE Nalgen®). The articular and bone samples were included prospectively, except for laboratory closing hours (21:00−7:30).

2.2. Bacteriological studies

SF and CSs were treated according to microbiological routine techniques. Bone samples were crushed using a bead mill (Retsch® MM400 crusher: frequency 30.0/s, for two min and 30 s). Tubes containing 10 sterile stainless steel beads (4 mm diameter) (AISI 304 Grade 1000; AFBMA; Hammer & Lemarié, France) in 10 mL of molecular biology grade distilled water were prepared, sterilized, tested and stored at room temperature for a maximum of 3 months in the laboratory. Following all safety protocols, contents of one tube was poured into each sterile container (HDPE) containing the OAS and grinded [4].

To ensure proper identification of cultures on solid media by MS, 50 µL of SF or CS were plated on Columbia agar supplemented with horse blood (5%) (Oxoid®), chocolate agar (Oxoid®) in atmosphere enriched with 5% CO_2 for 72 h and Columbia agar supplemented with horse blood (5%) in an anaerobic atmosphere for 5 days at 37 °C [18,19].

Each sample (n = 108) was enriched by inoculating 1 mL (minimum volume obtained for some joints) in an aerobic blood culture vial (BD BACTEC™ Plus Aerobic/F) and in an anaerobic blood culture vial (BD BACTEC™ Lytic/10 Anaerobic/F), incubated in automatic chambers for 14 days. Aerobic blood culture vial (BD BACTEC™ Plus Aerobic/F) and anaerobic blood culture vial (BD BACTEC™ Lytic/10 Anaerobic/F) were used because they proved to be the most efficient pair of blood aerobic/anaerobic culture media [20].

After extraction performed according to manufacturer's recommendations (Sepsityper® kit; Bruker), identification of bacterial species was performed using the MS technique (Microflex LT/SH mass spectrometer Biotyper, Bruker®) either on a single colony from agar media (routine use) [21] or on extracted enriched vial pellets (Sepsityper® kit), placed onto the polished steel target plate for rapid identification by MALDI-TOF. Once a positive vial was automatically detected, 1 ml of broth was extracted without delay (<2 h, to preserve spectra) with formic acid overlay [18] and analyzed via the same method as for colonies. Criteria for interpretation of results were based on the manufacturer's recommendations (Bruker®). Identification was established through biostatistics reliability levels on the basis of a correlation between the acquired spectrum and the reference spectra. The spectrum of the unknown test organism, acquired through MALDI Biotyper CA System Software®, was electronically transformed into the peak list. Using a biostatistical algorithm, this peak list was compared to reference peak lists of organisms in the reference database, and a log(score) value between 0.00 and 3.00 was calculated. The higher the log(score) value, the more reliable the degree of similarity (to a given organism in the reference FDA-cleared database). A log(score) value of ≥2.00 indicated an excellent probability for test organism

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