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The Role of Sonication in the Diagnosis and Treatment of Periprosthetic Infections in Orthopedic Surgery

Abstract of the Doctoral Thesis

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GENERAL PART

Chapter I. Periprosthetic Infections

Periprosthetic infections represent a major challenge in orthopedics, being associated with increased morbidity and significant costs. Sonication, through its ability to disassociate bacterial biofilms, offers a promising solution for improving the diagnosis and treatment of these infections. This thesis investigates the role of sonication in detecting and managing periprosthetic infections by analyzing the efficacy of this emerging technology.

1.1 Definition

Periprosthetic infections are severe infections associated with orthopedic implants that can arise from intraoperative contamination, postoperative bacteremia, or hematogenous dissemination. These infections affect approximately 1-2% of patients undergoing total arthroplasty, with higher rates in high-risk populations. Periprosthetic infections lead to significant disability and often require revision surgery or prosthesis explantation.

1.2 Epidemiology of PJIs in orthopedy and traumatology

The incidence of periprosthetic infections is influenced by the type of arthroplasty performed as well as by various individual risk factors, including advanced age, existing comorbidities (such as diabetes mellitus and obesity), and a history of previous infections. Generally, studies show that approximately 1-2% of patients undergoing total hip or knee arthroplasty develop periprosthetic infections. However, this percentage can be significantly higher in patients with additional risk factors such as smoking or the presence of immunodeficiencies.

Periprosthetic infections are caused by pathogenic microorganisms, most commonly bacteria such as Staphylococcus aureus, coagulase-negative Staphylococci (CNS), and streptococci. In some cases, these microorganisms can form biofilms, complex structures that protect the bacteria from the effects of antibiotics and the host's immune system, thereby complicating treatment and contributing to the persistence of the infection.

The infection rate also varies depending on the type of procedure: the incidence is approximately 1-2% for primary hip and knee arthroplasties but can increase to 3-5% in the case of prosthetic revisions. Factors such as prolonged surgical duration, significant intraoperative blood loss, and the use of multiple prosthetic devices are associated with an increased risk of infection.

1.3 Classification of Infections Associated with Orthopedic Pathology

Infections associated with orthopedic pathology are classified according to several critical factors, each playing a significant role in accurate diagnosis and the selection of appropriate treatment.

From the perspective of symptom duration, these infections can be categorized as acute, which appear rapidly and are usually severe; subacute, which develop over a longer period, typically between 3 and 12 months; and chronic, which persist for more than a year and develop slowly. The location of the infection is another classification criterion, distinguishing between periprosthetic infections, osteoarticular infections, and soft tissue infections, each type having different implications for treatment and prognosis.

The mechanism of onset, such as hematogenous, post-traumatic, or postoperative infections, provides information about how the infection was initiated and can influence therapeutic decisions.

Additionally, the classification of pathogens (Gram-positive bacteria, Gram-negative bacteria, anaerobes, and fungi) and the source of infection (intraoperative, early postoperative, late postoperative, or hematogenous) is essential for guiding specific treatment and preventing severe complications.

A deep understanding of these classifications is crucial for the effective management of infections within orthopedic pathology, thereby improving clinical outcomes and the quality of life for patients.

1.4 Etiology of Infections Associated with Orthopedic Pathology

The etiology of periprosthetic infections is diverse, involving a wide range of pathogens, including aerobic bacteria, anaerobic bacteria, and fungi, each contributing to the complexity of diagnosis and treatment.

Gram-positive bacteria, such as *Staphylococcus aureus* (including MRSA strains) and *Staphylococcus epidermidis*, are frequently involved and are known for their ability to form resistant biofilms. Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, present additional challenges due to their antibiotic resistance.

Anaerobic bacteria, such as *Peptostreptococcus* and *Clostridium* spp., are difficult to culture and often overlooked, but they play a significant role in the pathogenesis of infections.

Fungal infections, caused by *Candida* spp. and *Aspergillus* spp., are particularly problematic in immunocompromised patients, requiring advanced molecular diagnostics and specific antifungal treatments.

The management of these infections requires a multidisciplinary, personalized approach that considers the complexity of biofilms and the diversity of pathogens in order to improve prognosis and reduce the risk of recurrence.

1.5 Perioperative Antibiotic Prophylaxis: Pre-, Intra-, and Post-Operative

Perioperative antibiotic prophylaxis is crucial in preventing periprosthetic infections, aiming to reduce the risk of infection through the strategic administration of antibiotics around the time of surgery. This process involves a thorough assessment of the patient, including the treatment of any preexisting infection foci and screening for colonization by multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

The first-line antibiotic typically used is a first- or second-generation cephalosporin, such as cefazolin, due to its efficacy against both Gram-positive and Gram-negative bacteria. In cases of beta-lactam allergy, vancomycin or clindamycin is used as an alternative.

Preoperative prophylaxis involves administering the antibiotic 30-60 minutes before the surgical incision to ensure optimal concentrations at the time of incision. For antibiotics with longer administration times, such as vancomycin, the infusion should begin 90-120 minutes prior to surgery. Specific dosing varies: cefazolin is administered at a dose of 2 g intravenously in adults, with adjustments based on body weight, while vancomycin and clindamycin are dosed according to the patient's weight.

The administration of antibiotics should be limited to a maximum of 24 hours postoperatively to prevent adverse effects and the development of bacterial resistance, as there are no additional benefits from continuing treatment beyond this period.

Protocols based on international guidelines and adapted to local specificities contribute to the efficient administration of antibiotics, reducing infectious complications and improving the quality of care provided to patients.

1.6 Risk Factors for Periprosthetic Infections

Periprosthetic infections are a severe complication in orthopedic surgery, influenced by both intrinsic (patient-related) and extrinsic (surgery- and environment-related) risk factors.

Intrinsic risk factors include advanced age, obesity, diabetes mellitus, smoking, immunosuppression, malnutrition, and colonization with methicillin-resistant *Staphylococcus aureus* (MRSA).

Surgical risk factors involve prolonged surgery duration, surgical technique, instrument sterility, and the use of blood transfusions.

Perioperative factors such as hematomas, seromas, and surgical site infections also increase the risk of infection.

To assess the risk of complications, the Composite Risk Score (CRS) is used, which combines demographic, medical, surgical, perioperative, and microbiological factors into an overall score. This score aids in personalizing treatment and implementing appropriate preventive and therapeutic strategies, thereby reducing the risk of periprosthetic infections and improving clinical outcomes.

1.7 Pathogenesis of Periprosthetic Infections

The pathogenesis of periprosthetic infections involves several critical stages that contribute to the development and persistence of the infection. In the initial stage, bacteria may colonize the prosthesis through intraoperative contamination or hematogenous spread from other infectious sites. Once on the prosthetic surface, the bacteria adhere to extracellular matrix (ECM) proteins, such as fibronectin and collagen, using specific surface proteins known as MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules).

In the next stage, the adherent bacteria begin to produce a protective extracellular matrix, forming a biofilm. This biofilm shields the bacteria from the action of antibiotics and the host's immune cells, allowing them to survive under adverse conditions and persist over the long term. Bacteria within the biofilm can enter a state of slow growth or dormancy, thereby reducing the effectiveness of antibiotics, which primarily target rapidly dividing bacteria.

As the infection progresses, it can lead to chronic inflammation and the destruction of the bone tissue surrounding the prosthesis, a condition known as osteomyelitis.

At the molecular level, the bacteria involved in periprosthetic infections employ various mechanisms to support pathogenesis. *Staphylococcus aureus* produces exotoxins and enzymes such as hemolysins and proteases, which destroy host tissues and facilitate immune evasion. Additionally, pathogenic bacteria use secretion systems to inject effector proteins into host cells, modulating the immune response and promoting bacterial colonization and persistence.

Understanding these stages and molecular mechanisms is essential for developing effective strategies for the prevention, diagnosis, and treatment of periprosthetic infections, including targeting biofilms and modulating the host's immune response.

1.8 Diagnosis of Periprosthetic Infections

The diagnosis of periprosthetic infection is a complex process that is crucial for ensuring effective treatment. It involves a thorough clinical evaluation, laboratory investigations, and advanced imaging techniques. Patients may present with symptoms such as persistent pain, swelling, erythema, and wound discharge.

Blood tests, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), provide nonspecific indicators of inflammation but are essential for assessing the patient's condition. Synovial fluid analysis is critical, assessing the leukocyte count, differential leukocyte count, and bacterial cultures to confirm the infection.

Imaging techniques, including X-rays, CT scans, MRI, and PET, provide detailed information about the status of the prosthesis and adjacent tissues, helping to identify signs of infection.

Advanced microbiological techniques, such as sonication and polymerase chain reaction (PCR), are used to detect bacteria within the biofilm on prostheses, allowing for faster and more accurate diagnosis. Sonication helps to dislodge bacteria from the biofilm, facilitating their culture and identification, while PCR detects bacterial genetic material, even when cultures are negative. Differential diagnosis should rule out other causes of symptoms, such as non-infectious inflammatory reactions or rheumatologic conditions.

A multidisciplinary approach involving orthopedic surgeons, infectious disease specialists, and microbiologists is essential to ensure accurate diagnosis and appropriate treatment, thereby improving clinical outcomes for patients.

1.9 Therapeutic Management

The therapeutic management of periprosthetic infections combines surgical interventions with antibiotic treatment, tailored to the stage of infection, the pathogen involved, and the patient's characteristics. In Romania, both national and international protocols are applied.

For adults, initial empirical therapy may include vancomycin (15 mg/kg intravenously) or daptomycin (6 mg/kg intravenously) to cover MRSA, combined with third- or fourth-generation cephalosporins, such as ceftriaxone (2 g per day). In children, vancomycin is administered at doses of 10-15 mg/kg every 6 hours, and cefuroxime at doses of 50-100 mg/kg per day, divided into two or three administrations, with careful monitoring of renal and hepatic function.

Surgically, debridement and retention of the prosthesis may be considered in acute infections, combined with intensive antibiotic therapy. In chronic infections, a two-stage revision is preferred, involving the removal of the infected prosthesis, the use of an antibiotic spacer, and reimplantation of the prosthesis after the infection is eradicated. Antibiotic treatment typically lasts at least 6 weeks, with close monitoring of inflammatory markers and intraoperative cultures.

For elderly patients, therapies must be adapted to their comorbidities, with antibiotic doses adjusted to prevent toxicity, and continuous monitoring of renal and hepatic function. Oral antibiotic therapy may be considered in the consolidation phase of treatment. Long-term monitoring is essential to detect recurrences and evaluate clinical outcomes. A multidisciplinary approach, involving orthopedic surgeons, infectious disease specialists, and other healthcare professionals, is crucial for the success of treatment and the reduction of complications and recurrences.

Chapter 2: Sonication in the Context of Synovial Fluid

2.1 Definition

Sonication uses sound waves to disassociate bacterial biofilms and release microorganisms in a form accessible for microbiological analysis. This method is used to improve the detection of bacteria colonizing joint prostheses. The process involves removing the prosthesis, placing it in a container with a sterile solution, exposing it to ultrasound to dislodge the bacteria, followed by recovering and culturing the bacteria for identification. Sonication offers high diagnostic sensitivity, facilitating the rapid and accurate detection of bacteria, and is particularly useful in the diagnosis of chronic infections where biofilms are predominant.

The application of high-frequency sound waves allows more efficient recovery of bacteria, including those encapsulated in biofilms, which are often resistant to traditional culture methods without destroying them.

2.2 Current Uses and Sensitivity

Sonication has become essential in the diagnosis of periprosthetic infections due to its ability to disrupt bacterial biofilms, facilitating the release of microorganisms for microbiological analysis. By applying high-frequency sound waves, sonication enables more efficient recovery of bacteria, including those encapsulated within biofilms, which are often resistant to traditional methods.

Studies indicate that sonication significantly improves diagnostic sensitivity, detecting bacteria in 80-90% of periprosthetic infection cases, compared to 60-70% with standard culture methods. This underscores the importance of integrating sonication into diagnostic protocols for more effective management and treatment of periprosthetic infections.

2.3 Definition of Biofilm and Its Characteristics

A biofilm is a complex community of microorganisms embedded within a protective extracellular matrix composed of polysaccharides, proteins, and nucleic acids, which adheres to biotic or abiotic surfaces. The development of a biofilm occurs in several stages, beginning with the reversible adhesion of bacteria to a surface, followed by the formation of a mature biofilm containing microcolonies and water channels.

Biofilms provide bacteria with significant resistance to antibiotics and the host's immune response, protecting them and facilitating chronic infections. This resistance is due to the limited diffusion of antibiotics, enzymatic activity, and the presence of persister cells.

Biofilms are frequently involved in chronic infections, such as periprosthetic infections, and can form on implantable medical devices, complicating treatment. Understanding the formation and function of biofilms is crucial for developing effective prevention and treatment strategies for biofilm-associated infections, with major implications in clinical medicine and the management of medical devices.

2.4 Biofilm in the Pathogenesis of Periprosthetic Infections

The formation of biofilm on the surface of joint prostheses and other medical implants creates a protected environment for bacteria, allowing them to resist antimicrobial treatments and evade attacks from the host's immune system. The biofilm formation process begins with the initial adhesion of planktonic bacteria to the surface of the implant. Subsequently, the bacteria proliferate and produce an extracellular matrix composed of polysaccharides, proteins, and extracellular DNA, which cements the bacteria to the surface of the prosthetic implant. As the biofilm matures, the bacteria within it develop complex interactions. The host's immune response is also compromised by the presence of the biofilm. Immune cells, such as neutrophils and macrophages, have difficulty penetrating the biofilm matrix and eliminating the embedded bacteria.

Effective management of these infections requires not only surgical interventions to remove the biofilm and necrotic tissues but also antibiotic therapies that can penetrate the biofilm matrix and eradicate dormant bacteria.

2.5 Management of Biofilm-Associated Infections

Surgical treatment consists of aggressive debridement procedures necessary to eliminate the adherent biofilm on prostheses, but in severe cases, the entire prosthesis may need to be removed. Antibiotic treatment must be personalized based on microbiological culture results and sensitivity tests. Initial empirical therapy should include antibiotics active against the most common biofilm-forming bacteria, such as Staphylococcus aureus and Staphylococcus epidermidis. Generally, a 4-6 week antibiotic therapy is recommended, followed by consolidation therapy.

In addition to surgical interventions and antibiotic treatments, managing biofilm-related infections should include adjunctive measures such as glucose control and constant monitoring.

2.6 Antibiotic Resistance Mechanisms of Biofilm

Biofilm resists antibiotic treatments through the following mechanisms: protective extracellular matrix, metabolic heterogeneity and persister cells, efflux pump systems, resistance gene transfer, adaptive responses, and oxidative stress.

2.7 Prevention of Biofilm Formation in Periprosthetic Infections

Prevention strategies involve a multifactorial approach, including appropriate material selection using antibacterial materials with modified surfaces, aseptic surgical techniques, antibiotic prophylaxis, and wound irrigation. Technologies such as antibiotic coatings, dispersing agents, or ultrasonic technologies are also employed. Postoperative management involving wound care and careful monitoring is effective in preventing biofilm formation.

Preventing biofilm formation in periprosthetic infections requires a multifactorial approach that includes the appropriate selection of biomaterials, strict surgical techniques, and the use of advanced technologies and antibiofilm agents.

2.8 Syndromic Testing in Periprosthetic Infections

Syndromic testing is a modern approach to diagnosing periprosthetic infections, enabling the simultaneous detection of multiple pathogens and resistance markers from a single clinical sample. Utilizing advanced molecular biology techniques such as multiplex PCR, this method provides rapid and accurate diagnostics, outperforming traditional culture methods.

Syndromic testing can identify bacteria, fungi, and antimicrobial resistance genes, improving diagnostic sensitivity and reducing the risk of false-negative results. This approach is particularly effective in periprosthetic infections, where pathogens are often difficult to detect using traditional methods, and it allows for the rapid customization of therapy, thereby reducing diagnostic time and the risk of complications. Studies have shown that syndromic testing can detect pathogens in cases where standard cultures fail, demonstrating high sensitivity and specificity.

The implementation of this technology in clinical practice significantly improves outcomes for patients with periprosthetic infections.

2.9 The Use of Bacteriophages in the Treatment of Periprosthetic Infections

Bacteriophages are viruses that infect bacteria, destroying them through cell lysis or integrating their genetic material into the bacterial genome. They offer a promising alternative to antibiotics, being specific to certain pathogens and effective against antibiotic-resistant bacteria. Bacteriophages can also rapidly multiply within bacteria and can act synergistically with antibiotics. Recent studies have demonstrated the efficacy of phage therapy in treating periprosthetic infections, including those resistant to conventional treatments. However, challenges remain, such as the development of bacterial resistance to phages, inconsistent regulations, and difficulties in administration.

In conclusion, bacteriophages represent a valuable option for treating periprosthetic infections, but further research is needed to overcome current limitations and establish clear treatment protocols.

ORIGINAL PART

Chapter 3. Motivation for the Study and Working Hypothesis

Periprosthetic joint infections (PJI) represent a significant complication in orthopedic surgery, substantially impacting patients' quality of life and often leading to repeated surgical interventions and increased healthcare costs. Accurate and rapid diagnosis of these infections is crucial for effective treatment management. In this context, the sonication technique has emerged as a promising method for enhancing the detection of pathogens involved in periprosthetic infections.

The continuous increase in the number of arthroplasty procedures in recent years has led to a proportional rise in the incidence of periprosthetic infections. This scenario underscores the imperative need to develop and implement more efficient and rapid diagnostic methodsnal methods, such as joint aspiration and tissue cultures, have their limitations, particularly in detecting bacteria encapsulated within biofilms. Sonication, which uses ultrasonic waves to disrupt bacteria from biofilms, offers significant potential to improve the sensitivity and specificity of diagnosing periprosthetic infections.

The medical literature emphasizes the importance of timely and accurate PJI diagnosis to reduce associated morbidity and mortality. Delayed or incorrect diagnosis can lead to inadequate treatment, prolonging patient suffering and increasing medical costs. Therefore, it is essential to explore and validate new diagnostic methods that can provide superior results compared to traditional methods .

The central ***hypothesis*** of this study is that the use of sonication in diagnosing periprosthetic infections will lead to more accurate and rapid detection of pathogens compared to traditional methods. It is anticipated that sonication will enhance diagnostic sensitivity and specificity, thereby facilitating more targeted and effective treatment of periprosthetic infections. This hypothesis is based on sonication's ability to dislodge bacteria from biofilms, thereby facilitating their identification and effective treatment.

The study was conducted over the periods 2016-2018 and 2021-2023, involving a research team composed of orthopedic surgeons from the Bucharest Emergency Clinical Hospital and microbiologists from the National Institute of Infectious Diseases "Matei Balș" in Bucharest. The study's author collected sonication samples from patients hospitalized at the Bucharest Emergency Clinical Hospital, which were then sent to the

National Institute of Infectious Diseases "Matei Balș" where the sonication technique was available.

The biological materials used in this study were transported with utmost care from the Bucharest Emergency Clinical Hospital to the National Institute of Infectious Diseases "Matei Balș" to ensure the integrity and accuracy of the results. The transport process was carried out following strict biosafety protocols, using sterile and sealed containers to prevent any possible contamination. These containers were then placed in transport units equipped with controlled temperature maintenance systems, ensuring optimal conditions for the biological materials throughout the journey. The personnel responsible for transport were rigorously trained in the correct handling of biological samples, wearing appropriate personal protective equipment and following standardized procedures to avoid contamination.

Upon arrival at the institute, the materials were immediately received by the research team, which verified the integrity of the containers and samples, ensuring they had not been exposed to external factors that could compromise the validity of the experimental results.

The study included a cohort of 128 patients diagnosed through both joint aspiration and sonication, who were treated based on the results obtained through this advanced method.

Within the study, the collected samples underwent detailed microbiological analysis to identify pathogens and assess their antibiotic sensitivity. The sonication technique was employed to dislodge bacteria from biofilms, thus facilitating their detection through cultures and molecular methods. Comparing the results obtained through sonication with those obtained through traditional methods allowed for the evaluation of this technique's efficiency and accuracy in diagnosing periprosthetic infections.

The primary ***aim*** of this study is to assess the efficacy of the sonication technique, its limitations related to sample collection, processing, and interpretation, in diagnosing periprosthetic infections, and to compare the results with those of traditional methods. The study aims to determine whether sonication offers higher sensitivity in detecting infections and if this leads to more accurate and effective treatment. Additionally, the study seeks to evaluate the impact of sonication on clinical outcomes, including healing rates and the reduction of complications.

Through this study, it is intended to demonstrate that integrating the sonication technique into clinical practice and using it for processing collected fluid and histological samples can significantly improve the diagnosis and treatment of periprosthetic infections.

Positive results could transform current approaches, leading to standardized clinical protocols that include this innovative technique, making it mandatory practice for patients presenting with periprosthetic infections, as well as for aseptic patients, to enable the introduction of targeted antibiotic treatment, even in cases where clinical and routine culture data do not indicate an infection, and ultimately ensure the best outcomes for patients. Validating the working hypothesis will contribute to the development of more effective treatment strategies and improve the quality of life for patients with periprosthetic infections.

Chapter 4. Study Objectives

1. **Evaluating the Efficiency of Sonication in Diagnosing Periprosthetic Infections** The study compares the sensitivity and specificity of sonication with traditional microbiological culture methods. It analyzes the capability of sonication to release bacteria from biofilms formed on prosthetic surfaces, thus facilitating their identification through standard and advanced microbiological techniques. This helps determine the accuracy and reliability of sonication as a diagnostic method.
2. **Determining the Impact of Sonication on the Treatment of Periprosthetic Infections** The study evaluates the efficacy of antibiotic treatment administered based on results obtained through sonication and analyzes whether this reduces the recurrence rate of infections and improves the clinical outcomes of patients. This objective includes integrating sonication into treatment schemes to verify if it improves the management of infections initially considered aseptic.
3. **Investigating the Mechanisms of Bacterial Resistance in Biofilms Disassociated by Sonication** The study analyzes how sonication influences the expression of antibiotic resistance genes and the efficacy of antimicrobial treatments on bacteria released from biofilms. It also includes an evaluation of the genetic diversity and dynamics of bacterial populations in biofilms treated by sonication.
4. **Evaluating the Clinical Applicability of Sonication in Medical Practice** This objective involves an analysis of the costs and benefits, availability of the method, qualified personnel, and the practical impact of this technology. Barriers and limitations in implementing sonication in medical practice will be identified, and solutions for overcoming them will be proposed, thus contributing to the efficient integration of sonication into existing clinical protocols.
5. **Developing Standardized Guidelines and Protocols for Using Sonication** These guidelines will be developed based on the results obtained in the study and will include specific recommendations regarding the application of sonication, interpretation of results, and integration of this technology into clinical workflows. The guidelines will be adjusted according to available resources and implementation capacity in different territories.

Chapter 5. General Research Methodology

This chapter details the general methodology used in the research aimed at evaluating the efficacy of the sonication technique in diagnosing periprosthetic infections. The primary goal of the study is to compare this technique with traditional diagnostic methods and to assess its impact on treatment outcomes and clinical results.

Periprosthetic joint infections (PJI) are a significant complication in arthroplasty. Delayed diagnosis, unspecified etiology following joint aspiration, and empirical treatment are some of the factors that contribute to the chronicity of periprosthetic infections.

In Romania, these infections are often identified late due to several deficiencies in the healthcare system. These deficiencies include limited access to advanced diagnostic techniques, inadequate infrastructure for monitoring and managing periprosthetic infections, and insufficient continuous training programs for medical personnel involved in diagnosing and treating these infections. In many cases, joint aspiration and tissue cultures fail to identify the pathogens responsible for periprosthetic infections, leading to empirical, subjective treatments that may be ineffective, thereby contributing to the persistence and chronicity of the infection and the development of microbial resistance.

This situation is exacerbated by limited resources, insufficient understanding of how to use these resources effectively in relation to outcomes, and unequal access to advanced diagnostic equipment, such as sonication devices, which are available only in a few centers of excellence. The sonication technique could make a significant contribution to improving the diagnosis of periprosthetic infections in Romania.

Sonication enables the dislodging of bacteria from biofilms and their detection in collected samples, offering greater sensitivity and specificity compared to traditional methods. The widespread implementation of this technique could reduce the time required for identifying microorganisms and allow earlier initiation of targeted antimicrobial therapy, which could prevent the chronicity of infections and improve patient prognosis.

The study was designed as a retrospective observational study, monitoring the evolution of patients diagnosed with periprosthetic infections through traditional methods and sonication. The study periods were from May 2016 to December 2018, and from May 2021 to December 2023. The primary data collection site was the Bucharest Emergency Clinical Hospital, with biological material processing conducted at the National Institute of Infectious Diseases "Matei Balș" in Bucharest.

The study population consisted of 128 patients diagnosed with periprosthetic infections who had undergone arthroplasty. These 128 patients were diagnosed through both joint aspiration and sonication and were treated based on the results obtained through this advanced method.

Data were collected using the following methods:

- Synovial fluid samples: Collected via joint aspiration.
- Periprosthetic tissue samples: Collected during surgical interventions.
- Sonication technique: Used to dislodge bacteria from biofilms and facilitate their detection through microbiological and molecular methods.

The samples were processed and analyzed in the microbiology laboratory of the "Matei Balș" Institute. The sonication technique involves using ultrasonic waves to dislodge bacteria from prosthetic surfaces and release them into suspension, thereby facilitating their detection and identification through microbiological and molecular methods. The sonication procedure involves several specific steps:

- Sample collection: The samples are transferred into special sonication vials, containing a liquid medium suitable for dislodging bacteria.
- Application of ultrasonic waves: The vials with samples are placed in a sonication device, where they are exposed to high-frequency ultrasonic waves for a predetermined period. These waves create micro-cavities in the liquid, generating intense mechanical forces that dislodge the bacterial biofilm from prosthetic surfaces, sometimes risking bacterial membrane rupture.
- Centrifugation (vortexing): After sonication, the liquid samples are centrifuged to concentrate the dislodged bacteria.
- Microbiological and molecular analysis: The supernatant resulting from centrifugation is used for performing microbiological cultures and molecular tests, such as PCR (polymerase chain reaction), for the precise identification of pathogens and the evaluation of their antibiotic sensitivity.

The sonication technique is an innovative and advanced method, accessible only in a few centers in Romania, including the National Institute of Infectious Diseases "Matei Balș". The implementation and use of this technique were crucial for advancing the study and obtaining data comparable to those in the international literature.

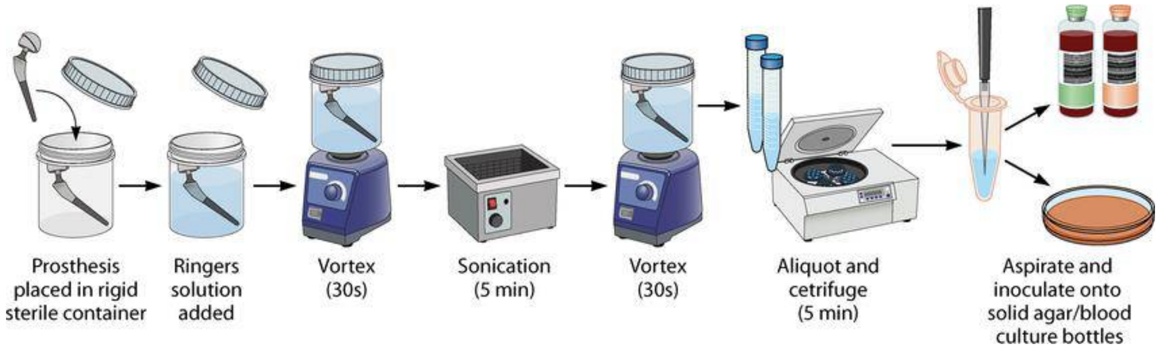

Image 5.1 Steps of Sonication

Inclusion Criteria for the Study:

- Patients with suspected periprosthetic infection.
- Patients who underwent hip or knee arthroplasty.
- Patients who provided informed consent to participate in the study.

Exclusion Criteria:

- Patients with periprosthetic infections diagnosed by methods other than joint aspiration.
- Patients with other types of infections not related to orthopedic prostheses.
- Patients who were unavailable for long-term follow-up or did not wish to participate in the study.

The progressive collection of documents related to the study subjects led to the need to integrate the information into an Excel database for subsequent statistical processing. The collected data were centralized in a database and statistically analyzed using IBM SPSS Statistics for Windows version 26.0 and Microsoft Excel Data Analysis. Operations included the systematization, grouping, and statistical analysis of quantitative and qualitative variables. The analyses included:

- **Descriptive Statistics:** Calculation of mean, median, mode, and standard deviation for quantitative variables.
- **Normality Tests:** Application of Kolmogorov-Smirnov and Shapiro-Wilk tests to verify the normal distribution of data.
- **Inferential Analyses:** Use of parametric and non-parametric tests, depending on data distribution, to compare results obtained through sonication with those obtained through traditional methods.

Study Limitations:

- **Sample Size:** Relatively small, which may affect the generalizability of the results.

- **Variability of Techniques:** Potential differences in the technique of sample collection and processing between different operators.
- **Single-Center Study:** Results might not be applicable to all medical centers.

Both quantitative (continuous or discrete) and qualitative (dichotomous or nominal) variables were included in the research plan. Descriptive and inferential statistics for each study were presented in detail. Nominal variables were analyzed using frequency tables and various graphical representations, including radial graphs, grouped or stacked columns, bar charts, scatter plots, line charts, and boxplots. For ordinal variables, measures such as mean, median, and mode were included. For quantitative variables, in addition to measures of central tendency (mean, median, mode) and dispersion (standard deviation), tests were applied to verify the normal distribution of the data, which is essential for choosing subsequent statistical tests, whether parametric or non-parametric.

This research plan was implemented in compliance with scientific, professional, and academic ethical standards, in accordance with the code of ethics and professional conduct of the following institutions: Carol Davila University of Medicine and Pharmacy, Bucharest Emergency Clinical Hospital, and the National Institute for Infectious Diseases "Matei Balş" in Bucharest.

The European Bone and Joint Infection Society (EBJIS), the Musculoskeletal Infection Society (MSIS), and the European Study Group for Implant-Associated Infections (ESGIAI) developed a set of criteria in 2021 for diagnosing PJI to provide clinicians with practical guidelines based on the most robust available evidence. The EBJIS criteria were developed through a comprehensive review of the literature, open discussions with society members and conference delegates, and evaluation by an expert group. These criteria were fully approved by EBJIS, MSIS, and ESGIAI. This process led to a three-tier diagnostic approach, providing a defined framework and guidelines that are useful for clinicians in daily practice.

Diagnostic Categories:

- **Unlikely Infection:** All tests are negative, suggesting or confirming the absence of infection.
- **Probable Infection:** Presence of a clinical sign or elevated CRP (C-reactive protein) level, along with another positive test (e.g., synovial fluid analysis, microbiology, histology, or nuclear imaging).

- **Confirmed Infection:** Any positive test from the confirmatory criteria (with very high specificity). If the patient presents with purulent puncture or a fistula tract, infection is present.

Tests Used and Their Significance:

- **Clinical Signs:** Ranging from acute septic arthritis to more subtle symptoms like pain or joint dysfunction.
- **Blood Biomarkers:** CRP and erythrocyte sedimentation rate (ESR). Elevated CRP levels (>10 mg/L) are associated with PJI in most cases but cannot be used alone to confirm or exclude infection.
- **Cytological Analysis of Synovial Fluid:** Synovial white blood cell count and the percentage of neutrophils are included in all major definitions. Proposed thresholds range from 1,500 to 4,000 cells/µL for leukocytes and 65% to 80% for PMNs.
- **Synovial Fluid Biomarkers:** Alpha-defensin has high specificity, with ELISA tests being more accurate than lateral flow tests.
- **Microbiology:** Preoperative synovial fluid cultures have low sensitivity in chronic infections. It is recommended to collect at least five reliable tissue samples during revision surgery.
- **Sonication:** Any positive culture from the sonicated fluid should be considered potentially infectious, with appropriate risks of potential contamination.
- **Histology:** Diagnosis is confirmed by the presence of five or more neutrophils in each of five high-power fields.
- **Nuclear Imaging:** Leukocyte scintigraphy is useful in diagnosing infected implants, especially when combined with bone marrow scintigraphy to reduce false positives.

Joint Aspiration or Arthrocentesis Procedure: A puncture needle (18-22 gauge) attached to a sterile syringe is used to aspirate synovial fluid from the joint space. Ultrasound guidance can be used to ensure correct needle placement. Once positioned in the joint, synovial fluid is aspirated, with the amount varying based on the joint pathology. The aspirated fluid is transferred to sterile containers for microbiological, cytological, and biochemical analysis. If infection is suspected, part of the fluid can be sent for culture and antibiotic sensitivity testing. All procedural details, including the amount and characteristics of the aspirated fluid, are documented in the patient's record.

Sonication Procedure: Extracted osteoarticular implants are placed in sterile containers in the operating room and immediately sent for sonication to the National Institute for

Infectious Diseases “Matei Balș” - Microbiology Laboratory. In a laminar airflow hood, sterile 0.9% saline is added to cover at least 90% of the implant, which is then vortexed. The container with the sample is placed in an ultrasonic bath at a frequency of 42 kHz (BactoSonic®14.2, Bandelin GmbH, Berlin, Germany) for 1 minute. After sonication, the sample is vortexed again and processed in the laminar airflow hood. The sonicated fluid is quantitatively cultured on solid media (100 µl each) and in enrichment liquid medium (3-4 ml). The solid media used include blood agar, lactose agar (ThermoFisher Scientific™-Oxoid, Germany), and chocolate agar (bioMérieux S.A., France), in duplicate. One set of blood agar, chocolate agar, and lactose agar plates are incubated in a CO2-enriched atmosphere, while another set of blood and chocolate agar plates are incubated anaerobically using reducing agent pouches. Plates are incubated at 37°C and read daily for up to 5 days. The enrichment liquid medium (thioglycolate broth, bioMérieux S.A., France) is incubated at the same temperature for up to 14 days, with bacterial growth monitored by observing changes from clear to turbid medium.

Reading and Interpretation: Cultures grown on solid media are identified using the MALDI Biotyper® system (Bruker Daltonics GmbH & KG, Bremen, Germany). Clinically significant results are >= 50 CFU/ml. Results between 1-40 CFU/ml are interpreted in conjunction with clinical data. If only enrichment media cultures are positive (thioglycolate broth), the result is usually not relevant (likely contamination during extraction, transport, or processing), except in cases of positive anaerobic cultures and patients on antibiotic treatment. In these situations, further investigations are required, such as cultures from periprosthetic tissue, joint aspirate cultures, histopathological examination, intraoperative data, etc. Antibiotic and antifungal sensitivity testing is performed immediately after identifying the infectious agent using the Vitek® 2 Compact (bioMérieux S.A., Marcy l’Etoile, France) or the Micronaut system (MERLIN Diagnostika GmbH, Bornheim, Germany), and interpretation is according to the EUCAST guidelines.

Chapter 6. Prevalent Pathogens in Periprosthetic Infections: Analysis of the Distribution of Various Bacterial Strains in Periprosthetic Infections

6.1 Introduction

This chapter aims to analyze the distribution of different bacterial strains isolated in periprosthetic infections. We will evaluate the prevalence of these bacteria and investigate the predominant strains, comparing them based on various clinical and demographic variables of patients and the type of diagnostic investigation used.

6.2 Materials and Methods

This study was designed as a retrospective analysis, focusing on patients diagnosed with periprosthetic infections. The primary objective was to evaluate the prevalence and types of bacteria isolated in these infections using both traditional joint aspiration techniques and the advanced sonication method. The study was conducted over two different periods: from 2016 to 2018 for Group A, and from 2021 to 2023 for Group B, involving patients who underwent joint aspiration and sonication and were admitted to the Orthopedics Clinic of the Bucharest Emergency University Hospital. The study included a total of 128 patients diagnosed with periprosthetic infections through joint aspiration combined with the sonication technique and treated based on the results obtained through this method.

The treatment of these patients was adjusted based on the results obtained from both diagnostic methods.

6.3 Results

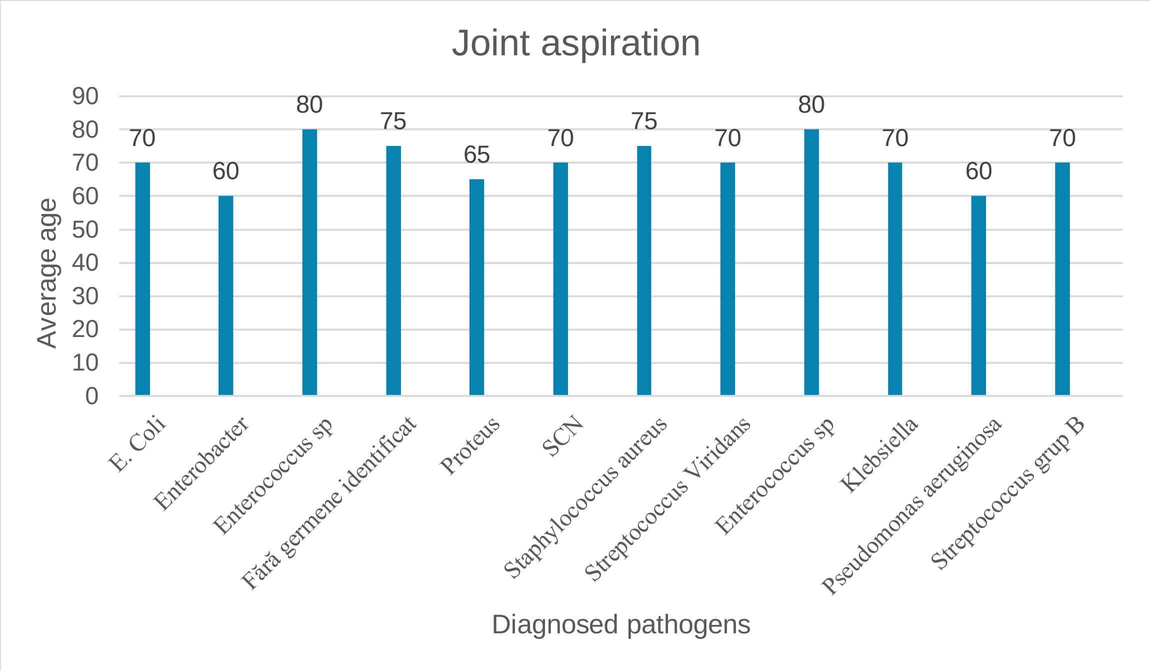

Figure 1. Distribution of Microorganism Types Identified through Joint Aspiration – Group A

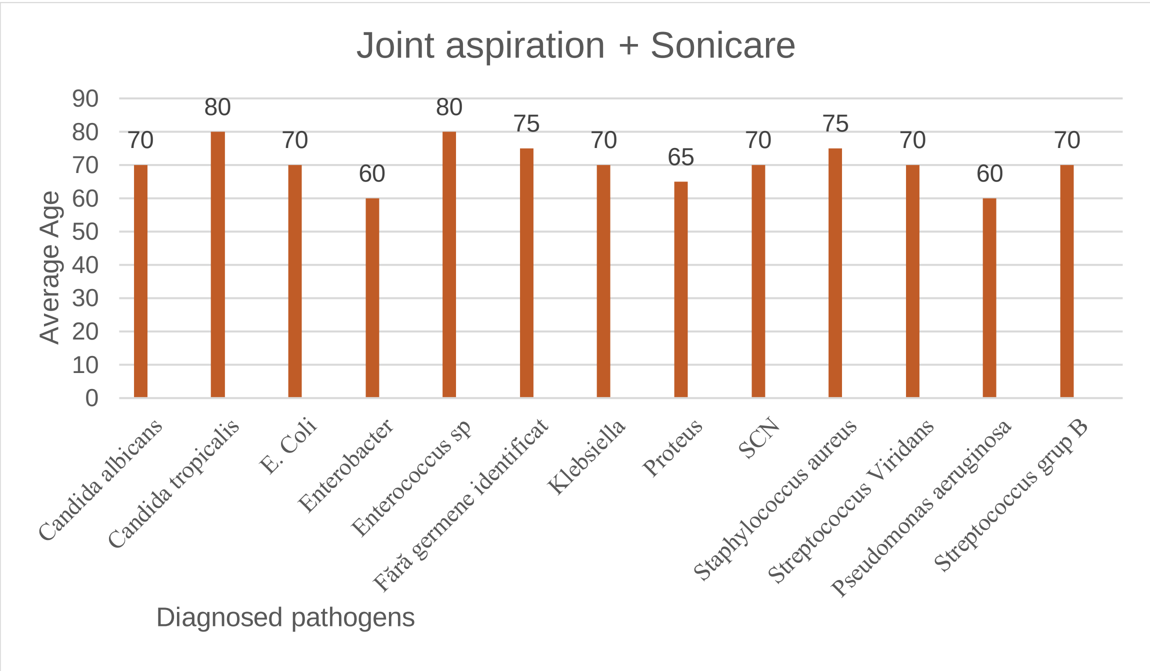

Figure 2. Distribution of Microorganism Types Identified through Joint Aspiration and Sonicare – Group A

Figures 1 and 2 illustrate the age distribution of Group A patients diagnosed through joint aspiration and sonication. The similar distribution observed within the age range of 50-70 years suggests that both diagnostic methods are effective in detecting infections in these age groups, while also indicating a higher prevalence of periprosthetic infections within these categories.

The prevalence calculated for the joint aspiration diagnostic method reveals that aerobic Gram-positive cocci are the most common pathogens, with a prevalence of 40.63%, followed by patients with no identified pathogen, at a prevalence of 34.38%. Conversely, pathogens such as Staphylococcus lentus, Corynebacterium, and fungi from the genus Candida have a null prevalence.

In contrast, the sonication method appears more effective in detecting infections in certain age groups, such as 75 and 81 years old. Analysis of the prevalence of infections diagnosed through joint aspiration plus sonication showed that aerobic Gram-positive cocci were the most frequently identified pathogens, with a prevalence of 57.81%. Additionally, coagulase-negative staphylococci (CNS) and aerobic Gram-negative bacilli demonstrated notable prevalences, each at 31.25%. The sonication method also proved effective in detecting less common pathogens, such as Enterococcus species with a prevalence of 7.81%, and fungi from the genus Candida (including Candida albicans and Candida tropicalis), each with a prevalence of 1.56%.

The distribution shows a peak at the age of 80 years among patients diagnosed with periprosthetic infections using the joint aspiration method, indicating a more frequent use of this method in older patients. In contrast, the sonication method shows a concentration at ages 75 and 80 years, suggesting increased efficacy in detecting periprosthetic infections in elderly patients when sonication is also employed. For younger patients, joint aspiration seems to be used more frequently compared to sonication.

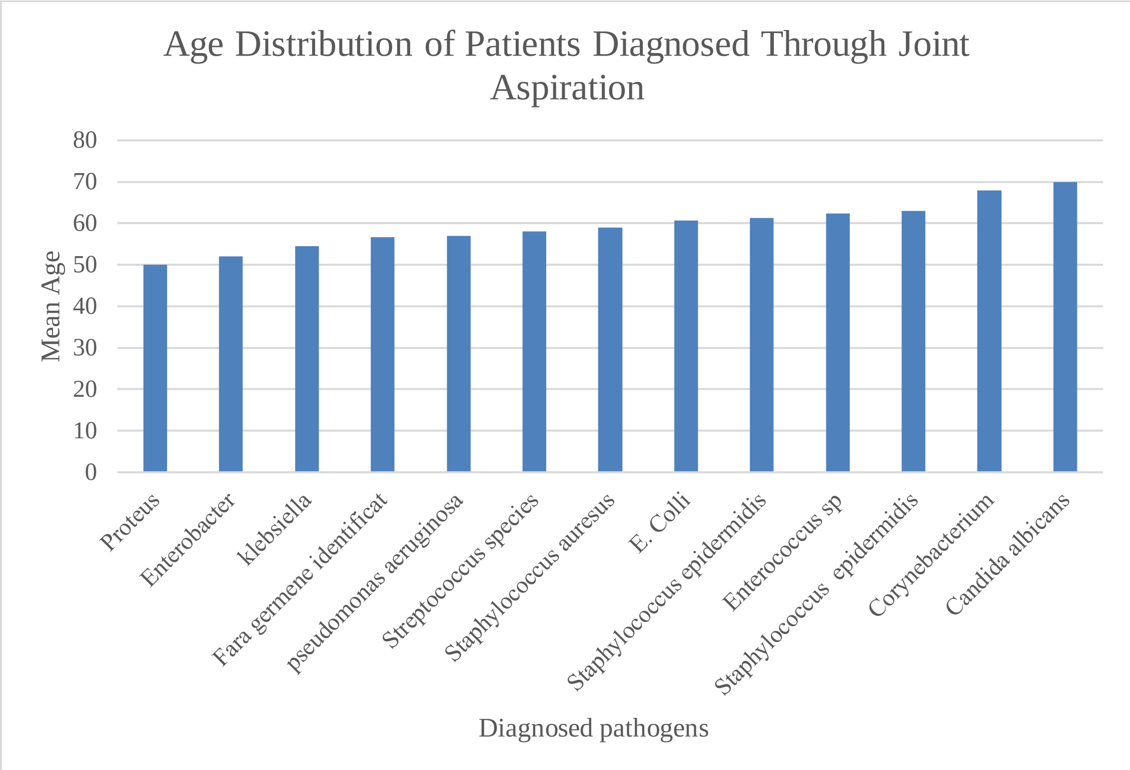

Figure 3. Age Distribution of Patients Diagnosed Through Joint Aspiration – Group B

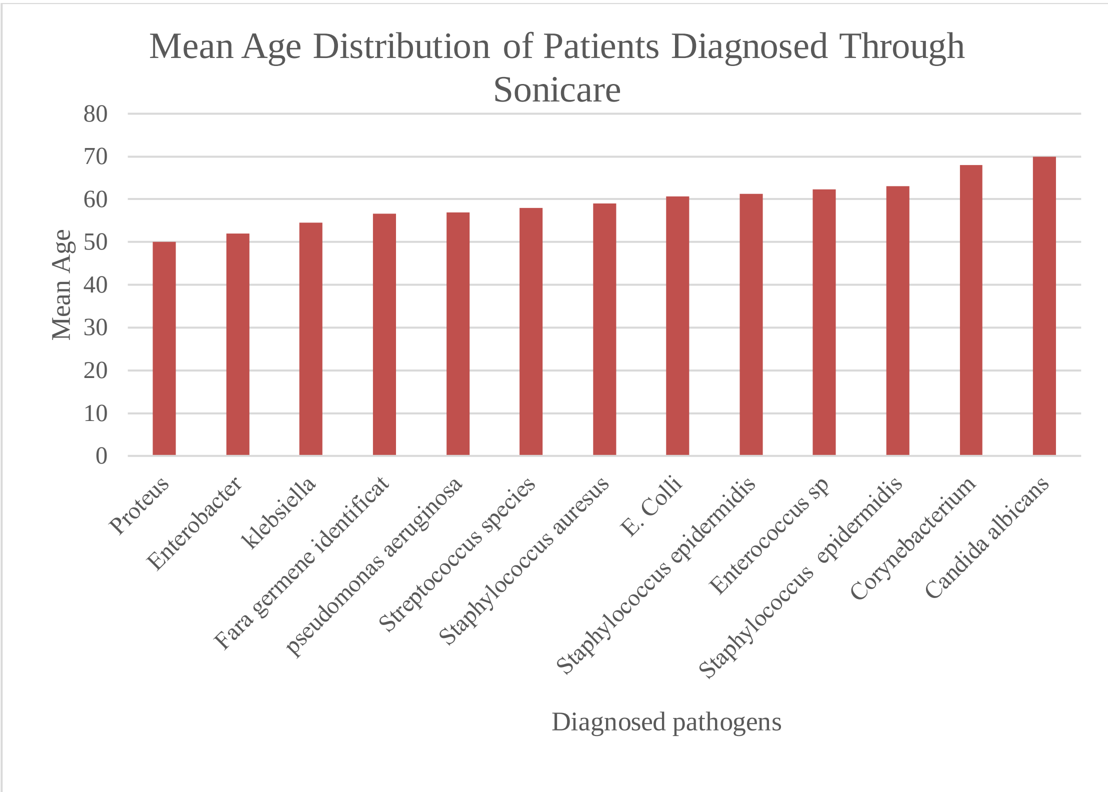

Figure 4. Age Distribution of Patients Diagnosed Through Joint Aspiration and Sonicare – Group B

Figures 3 and 4 illustrate the age distribution of patients in Group B, diagnosed through joint aspiration and sonication, respectively. The similar distribution within the 55-75 year age range suggests that both diagnostic methods are effective in detecting periprosthetic infections within these age groups, reflecting a higher prevalence of infections in this segment of the population.

The prevalence calculated for the joint aspiration diagnostic method indicates that aerobic Gram-positive pathogens, particularly Staphylococcus aureus and Staphylococcus epidermidis, are the most frequently identified pathogens, with notable prevalence rates. In contrast, pathogens such as Corynebacterium and fungi of the genus Candida exhibited low prevalence, suggesting a rare occurrence in this specific context.

Sonicare has proven effective in detecting less common pathogens, such as Enterococcus species and fungi of the genus Candida, each with a notable, though lower, prevalence compared to the major pathogens. Additionally, Escherichia coli and Klebsiella spp. displayed significant prevalence rates, highlighting the diversity of pathogens that can be detected through this method.

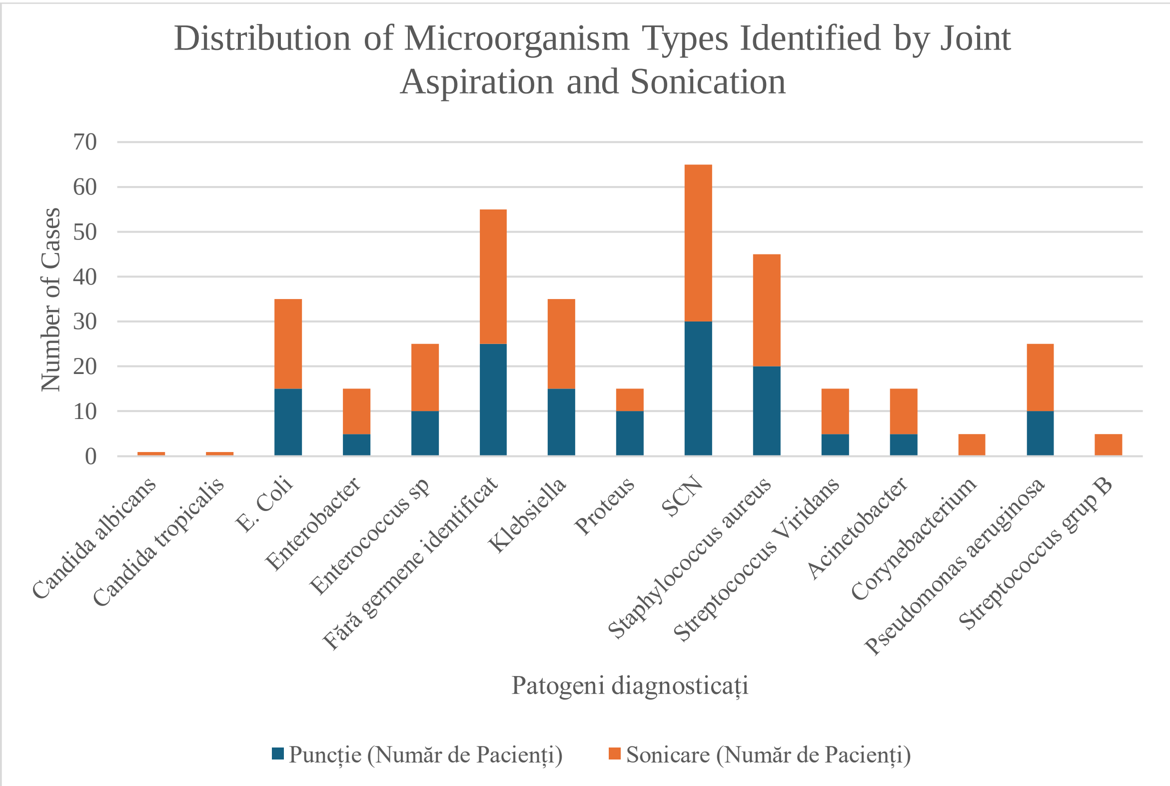

Figure 5: Distribution of Microorganism Types Identified by Joint Aspiration and Sonication – Group A

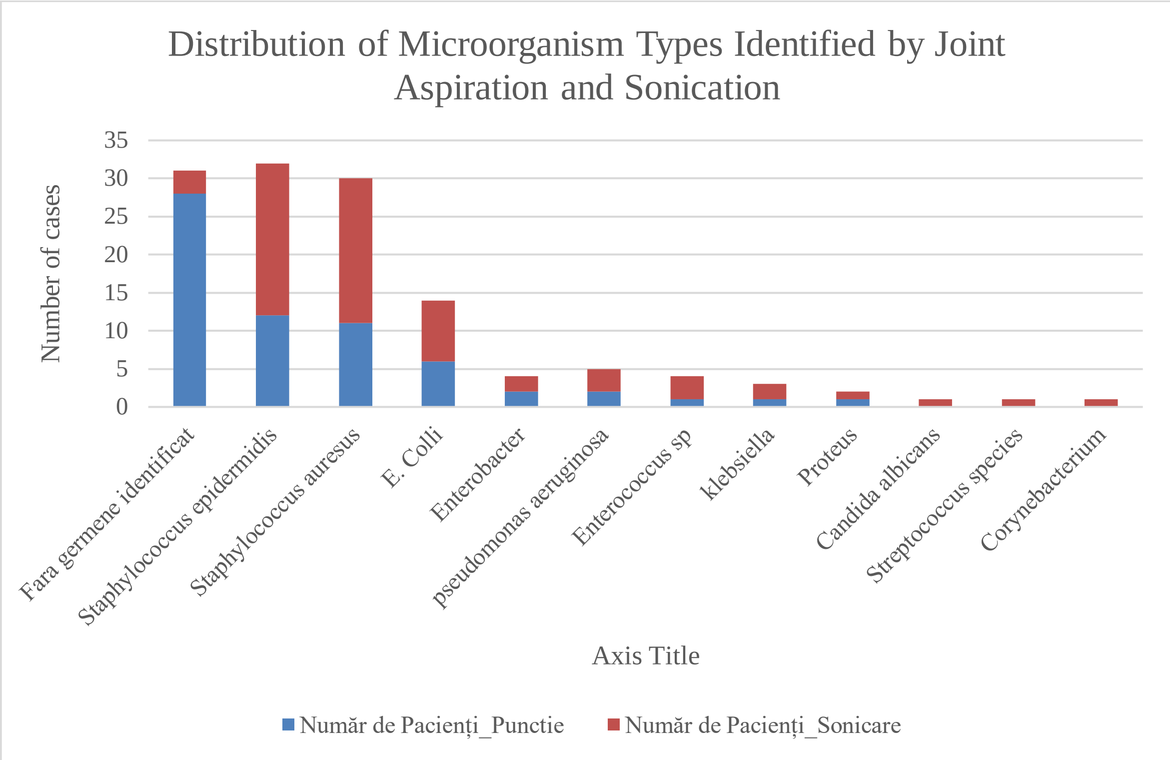

Figure 6: Distribution of Microorganism Types Identified by Joint Aspiration and Sonication – Group B

Figures 5 and 6 illustrate the distribution of microorganism types identified by joint aspiration and sonication for Groups A and B of patients.

Staphylococcus epidermidis is the most frequently identified bacterium in both methods, with slightly different percentages: 23.44% in joint aspiration and 31.25% in sonication.

Staphylococcus aureus is more frequently detected through sonication (14.06%) compared to joint aspiration (9.38%), suggesting a higher sensitivity of the sonication method for this pathogen.

In patients diagnosed via joint aspiration, 32.81% of samples did not identify any specific microorganism, indicating limitations of this method in detecting certain pathogens. In contrast, in patients diagnosed through sonication, the percentage of samples without an identified pathogen is 20%, highlighting the greater sensitivity of the sonication method in identifying microorganisms.

Other Gram-negative bacteria, such as E. coli and Klebsiella, are present in both methods, but again, differences in prevalence are observed. E. coli represents 7.81% in joint aspiration cases, compared to approximately 7% through sonication.

A statistical analysis was conducted to compare the distribution of pathogens identified by joint aspiration and sonication in Group B (Figure 6.14) using the Chi-square test. The results indicated a Chi-square (χ^2) value of 29.25, associated with a p-value of 0.0036. This p-value, statistically significant at a 0.05 significance level, suggests the presence of relevant differences between the distributions of pathogens identified by the two methods.

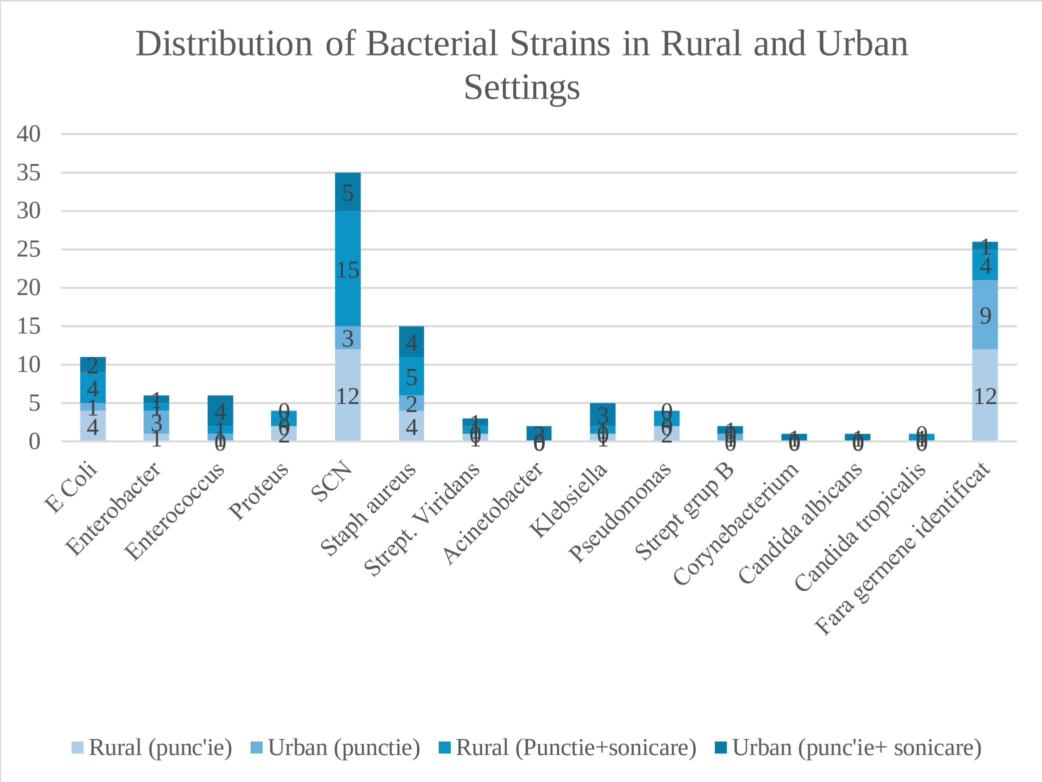

Figure 7: Distribution of Bacterial Strains in Rural and Urban Settings Among Patients with Joint Aspiration and Sonication – Group A

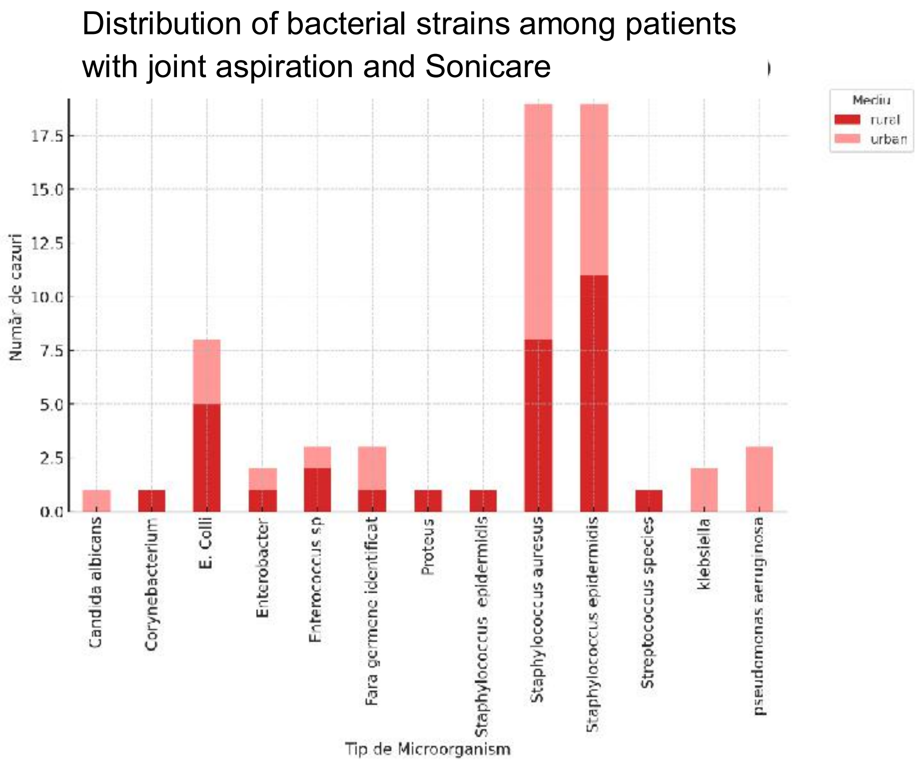

Figure 8: Distribution of Bacterial Strains in Rural and Urban Settings Among Patients with Joint Aspiration and Sonication – Group B

In patients diagnosed through joint puncture, the distribution of bacterial strains in rural areas shows a predominance of bacteria such as coagulase-negative Staphylococcus, Staphylococcus aureus, and E. coli. In urban areas, the distribution is more diverse, but coagulase-negative Staphylococcus and Staphylococcus aureus remain predominant. For patients diagnosed using sonication, the distribution of bacterial strains in rural areas demonstrates a greater diversity of microorganisms. In urban settings, the bacterial distribution is more uniform, with notable presence of gram-negative bacteria such as E. coli and Klebsiella, as well as fungi from the Candida family. Comparing the two diagnostic methods, sonication proves to be more effective in identifying a wider range of microorganisms, including fungi. The percentage of samples without an identified pathogen is lower with sonication, suggesting higher sensitivity and a better capacity to detect pathogenic bacteria and fungi.

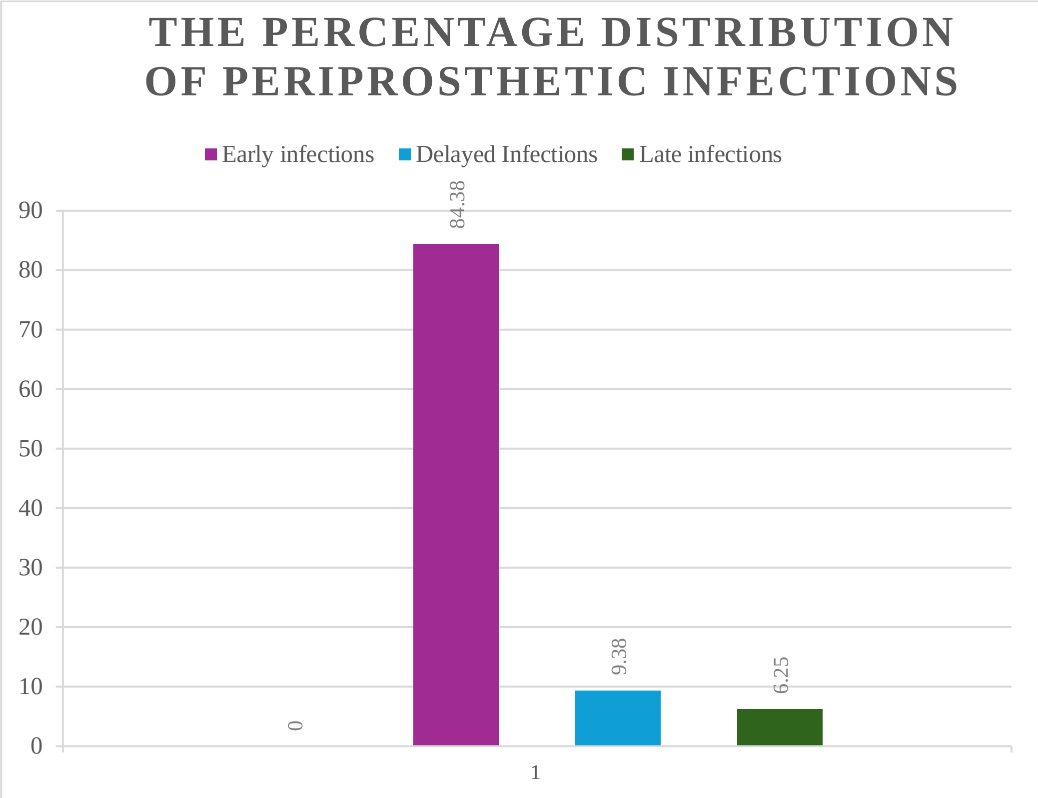

Figure 9: Percentage Distribution of Early, Delayed, and Late Infections

Figure 9 illustrates the percentage distribution of periprosthetic joint infections (PJI), categorized into three main groups: early infections, delayed infections, and late infections.

Early infections constitute the largest proportion, accounting for 84.38% of all cases. These infections typically occur within the first three months postoperatively.
Delayed infections, occurring between 3 and 12 months after surgery, make up 9.38% of the cases. These infections can be more challenging to diagnose and treat.

Late infections, which develop more than one year after the prosthesis implantation, represent 6.25% of the cases. These infections are often associated with the hematogenous spread of bacteria from other infection sites within the body.

The percentage distribution highlights that early infections are the most common.

In conclusion, sonication demonstrates superior efficacy and accuracy in diagnosing periprosthetic joint infections by detecting a broader range of microorganisms, including anaerobes and difficult-to-culture strains. The precise diagnosis achieved through sonication allows for targeted antibiotic treatment, reducing the need for empirical therapies and lowering the risk of infection recurrence. By facilitating a more accurate assessment of bacterial antibiotic sensitivity, sonication contributes to the rational use of antibiotics and helps limit the development of bacterial resistance. Additionally, sonication provides more precise data for monitoring inflammation and adjusting treatment, thereby improving the management of periprosthetic infections and clinical outcomes for patients. The use of sonication, in combination with surgical interventions and rehabilitation treatments, can significantly enhance patient prognosis and quality of life.

These conclusions underscore the need for widespread adoption of sonication as a diagnostic method in clinical practice to ensure more effective diagnosis and management of periprosthetic joint infections.

6.4 Discussion

Periprosthetic joint infections (PJI) represent a significant challenge in orthopedics, having a considerable impact on both patients and healthcare systems. Sonication is an innovative technology that enhances the diagnosis of these infections by disrupting bacterial biofilms and releasing microorganisms for more accurate identification. Studies indicate that sonication detects a wider variety of bacteria, including difficult-to-culture strains and anaerobes, providing a more comprehensive understanding of the pathogens involved in PJIs.

Comparative analysis between sonication and simple joint aspiration reveals the superiority of sonication in detecting infections across patients of all ages and from various backgrounds. Sonication also shows better correlation with inflammatory markers such as CRP, ESR, leukocyte count, and fibrinogen, suggesting a superior ability to detect severe infections.

Sonication enables the initiation of targeted antibiotic therapy, reducing the risk of recurrence and preventing the development of bacterial resistance. Additionally, it improves patient survival rates and decreases the need for repeated surgical interventions, offering a better long-term prognosis.

The widespread implementation of sonication in clinical practice could standardize the diagnosis of periprosthetic infections, significantly enhancing the quality of patient care and reducing the costs associated with prolonged treatments and complications.

Chapter 7. Comparison of Diagnostic Methods in Detecting Periprosthetic Infections: Evaluation of Accuracy and Efficacy of Different Microbiological and Imaging Diagnostic Methods

7.1. Introduction

Periprosthetic infections are severe and complex complications of total knee and hip arthroplasty, leading to intense pain, loss of joint function, and the need for additional surgical interventions such as prosthetic revisions. These infections deeply affect patients' physical health, quality of life, and psychological well-being. The incidence of infections varies between 1-2% for primary arthroplasty but can reach 5-10% in revision arthroplasties. Early and precise diagnosis is essential to prevent severe complications and ensure effective treatment. Diagnostic methods include clinical, microbiological, and imaging approaches, each with specific advantages and limitations.

In recent decades, microbiological and imaging diagnostic methods have evolved significantly. Traditional bacterial culture, though considered the gold standard, has limitations such as low sensitivity in chronic infections and the long time required to obtain results. Advanced methods such as implant sonication and polymerase chain reaction (PCR) offer advantages in terms of sensitivity and specificity but require specialized equipment and expertise. Imaging methods, including standard radiography, bone scintigraphy, magnetic resonance imaging (MRI), and positron emission tomography-computed tomography (PET-CT), are essential for evaluating infection extent and planning surgical treatment.

Accurate and rapid diagnosis is crucial for guiding therapeutic decisions and optimizing clinical outcomes. Early identification of pathogens and assessment of infection extent allow more effective surgical interventions and better-targeted antibiotic treatments. The choice between one-stage and two-stage revision largely depends on accurate infection diagnosis and the clinical conditions of the patient. This meta-analysis compares the accuracy and efficacy of different microbiological and imaging diagnostic methods, providing a comprehensive synthesis to support clinical decisions and improve patient outcomes.

7.2. Materials and Methods

In this work, we will address the following aspects:

1. **Microbiological Diagnostic Methods:** We will discuss bacterial culture, implant sonication, and PCR, evaluating the advantages and limitations of each method.
2. **Imaging Diagnostic Methods:** We analyzed standard radiography, bone scintigraphy, MRI, and PET-CT, highlighting their applicability and efficacy in the context of periprosthetic infections.
3. **Comparison of Diagnostic Methods:** We directly compared microbiological and imaging methods using relevant data from articles to evaluate their relative accuracy and efficacy.
4. **Clinical Implications and Recommendations:** We will discuss the practical implications of our results and formulate recommendations for clinicians based on the best available evidence.

Importance of the Study

Comparative studies demonstrate the efficacy of various treatment strategies for periprosthetic infections. This study showed that one-stage revision can be as effective as two-stage revision for infected total knee arthroplasty if patients are carefully selected. Accurate diagnosis and postoperative monitoring are crucial for optimal clinical outcomes. This meta-analysis aims to comprehensively evaluate microbiological and imaging diagnostic methods to improve clinical practices and patient outcomes. A multimodal approach combining microbiological and imaging methods can optimize therapeutic decisions, reducing costs and surgical stress. Future research should focus on multicenter randomized studies to establish clear guidelines for diagnosing and treating periprosthetic infections.

Study Objective

Periprosthetic infections pose a major challenge in orthopedics, with a significant impact on patients and the healthcare system. Early and precise diagnosis of these infections is essential to ensure effective treatments and prevent severe complications. Given the complexity and importance of this issue, the primary objective of this study is to evaluate and compare the accuracy and efficacy of different microbiological and imaging diagnostic methods in detecting periprosthetic infections.

Study Design

This meta-analysis was conducted to evaluate and compare the accuracy and efficacy of different microbiological and imaging diagnostic methods in detecting periprosthetic infections. We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure the rigor and transparency of the review process. {100}

To conduct a systematic review and identify all relevant studies, we performed a comprehensive search in the following databases: PubMed, Cochrane Library, Embase, and Google Scholar

Inclusion Criteria

1. **Type of Study:** Randomized clinical trials, observational studies, and cohort studies that evaluated microbiological and imaging diagnostic methods for periprosthetic infections.
2. **Study Population:** Patients undergoing total knee or hip arthroplasty with suspected or confirmed periprosthetic infection.
3. **Diagnostic Methods:** Evaluation of microbiological methods (bacterial culture, implant sonication, PCR) and imaging methods (standard radiography, bone scintigraphy, MRI, PET-CT).
4. **Reported Outcomes:** Studies that provided sufficient data for calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Exclusion Criteria

1. **Type of Study:** Case studies and case series without control groups that do not provide sufficient comparative data.
2. **Data Quality:** Studies with incomplete or insufficient data for statistical analysis that do not allow calculation of diagnostic performance indicators.
3. **Language:** Studies published in languages other than English to ensure consistency and accessibility of the analysis.
4. **Study Population:** Studies that did not include patients with total knee or hip arthroplasty to maintain the specificity of the meta-analysis.

7.3 Results

After applying the inclusion and exclusion criteria and the study selection process, we included a total of 25 studies in this meta-analysis. These studies evaluated various microbiological and imaging diagnostic methods for detecting periprosthetic infections.

Characteristics of the Studies

1. **Microbiological Diagnostic Studies:**
    - **Bacterial Culture:** 10 studies evaluating the sensitivity and specificity of bacterial culture in identifying pathogens from periprosthetic infections.
    - **Implant Sonication:** 7 studies analyzing the efficacy of sonication compared to traditional culture methods.
    - **PCR:** 5 studies investigating the role of PCR in the rapid diagnosis of infections.
2. **Imaging Diagnostic Studies:**
    - **Standard Radiography:** 6 studies evaluating the utility of radiography in detecting early signs of infections.
    - **Bone Scintigraphy:** 5 studies analyzing the sensitivity and specificity of bone scintigraphy.
    - **MRI:** 8 studies investigating the capacity of MRI to provide detailed images of soft tissues and bone structures.
    - **PET-CT:** 4 studies evaluating the efficacy of PET-CT in detecting increased metabolic activity associated with infections.

7.4 Discussion

PET-CT demonstrated the best performance in this meta-analysis, with both sensitivity and specificity of 92.5%. This suggests that PET-CT not only correctly identifies patients with periprosthetic infections but also minimizes the number of false-positive results. The efficacy of PET-CT can be attributed to its ability to detect increased metabolic activity associated with infections, thus providing a clear and precise image of the affected areas.

Implant sonication also showed remarkable performance with a sensitivity of 85% and specificity of 97.5%. This method is particularly useful for detecting bacteria in biofilms, which are often difficult to identify through traditional methods. The high sensitivity indicates a high probability of detecting infections, while the nearly perfect specificity significantly reduces the risk of false-positive results.

Recommendations for Future Research

Future research should focus on conducting randomized and multicenter clinical trials to evaluate the comparative performance of diagnostic methods in various patient populations. It would also be useful to develop standardized protocols for diagnosing periprosthetic infections to reduce variability between clinical studies and improve the comparability of results.

This meta-analysis highlights the importance of using modern diagnostic methods in detecting periprosthetic infections. PET-CT and implant sonication have proven to be the most effective methods, offering high values for sensitivity and specificity. Integrating these methods into clinical practice can significantly improve patient outcomes, ensuring early and accurate diagnosis and allowing the prompt initiation of appropriate treatment.

7.5 Patients' Guide: Postoperative Monitoring and Follow-Up for Early Infection Detection

After undergoing joint replacement surgery (hip, knee), it is crucial to remain vigilant and follow specific measures and recommendations to quickly identify any signs of infection. Periprosthetic infections, if not treated promptly, can lead to serious complications. Here is a guide outlining the steps you should follow and the signs you need to watch for:

1. Monitoring Your General Condition

- **Fever:** A body temperature above 38°C (100.4°F) that persists for more than 24-48 hours postoperatively should be reported to your doctor immediately.
- **General Weakness:** Extreme fatigue, loss of appetite, or a general feeling of illness may be signs of an infection.

2. Inspecting the Surgical Site

- **Redness and Swelling:** Watch for any increase in redness, swelling, or warmth around the operated area. These may indicate inflammation or infection.
- **Drainage:** If you notice discharge from the incision site that is yellow, green, or has an unpleasant odor, contact your doctor immediately.
- **Pain:** Pain is normal after surgery, but if you experience a sudden increase in pain intensity or pain that does not improve with prescribed analgesics, it could be a sign of infection.

3. Caring for the Incision

- **Keep the Incision Clean and Dry:** Follow your doctor's instructions regarding dressing changes and incision site hygiene.
- **Avoid Activities That Could Affect the Incision:** Refrain from lifting heavy objects, making sudden movements, or rubbing the operated area.

4. Respiratory or Urinary Symptoms

- **Persistent Cough, Difficulty Breathing:** These could indicate a systemic infection or a postoperative complication.
- **Pain or Difficulty Urinating:** Urinary tract infections can occur and may worsen your overall condition.

5. Report Any Suspicious Symptoms to Your Doctor Immediately

- **Do Not Delay Medical Consultation:** If you have any doubts about the symptoms you are experiencing, it is essential to contact your doctor as soon as possible. Periprosthetic infections require prompt intervention to prevent prosthesis damage and other complications.

6. Schedule Regular Follow-Up Visits

- **Postoperative Consultations:** Attend all scheduled appointments to ensure proper monitoring of healing and early detection of any signs of infection.
- **Follow-Up Tests:** Your doctor may occasionally recommend blood tests or additional imaging to monitor the healing process.

7. Maintain Open Communication with Your Medical Team

- **Questions and Clarifications:** Do not hesitate to ask for clarifications about any symptoms you notice or the steps to follow in postoperative care.

This guide is designed to help you effectively monitor your postoperative recovery and detect any potential complications early. Prompt communication with your doctor can make the difference between a successful recovery and serious complications.

7.6. Diagnostic Algorithm for Periprosthetic Joint Infections (PJI)

This meta-analysis has helped in the creation of an effective and practical diagnostic algorithm for periprosthetic joint infections (PJI). It is important to integrate the latest guidelines and recommendations from the medical literature. Here is a proposed algorithm based on the most recent research and clinical practices:

1. **Initial Clinical Evaluation:**
    - **Symptoms and Clinical Signs:** Assess the patient for persistent pain, swelling, localized warmth, and erythema around the prosthesis. The presence of a sinus tract or drainage may be a clear indication of infection.
2. **Initial Serological Tests:**
    - **ESR, C-reactive protein, Fibrinogen, White Blood Cell Count:**
        - If inflammatory markers are elevated, the suspicion of PJI is high. These inflammatory markers are the first steps in the algorithm, as they have good sensitivity for detecting PJI.
3. **Joint Aspiration:**
    - **Arthrocentesis:** If serological tests are positive or there is a strong clinical suspicion of PJI, aspiration of the affected joint is recommended.
    - **Synovial Fluid Analysis:** Evaluate the white blood cell count, percentage of neutrophils (PMNs), and perform microbial cultures. Recently, alpha-defensin testing has shown high sensitivity for PJI and can be included if available.
4. **Imaging Studies:**
    - **Advanced Imaging:** Plain radiography may show indirect signs of infection, but for higher suspicion, magnetic resonance imaging (MRI) or computed tomography (CT) can be used to detect fluid collections or subtle bone changes.
5. **Tissue Biopsy and Periprosthetic Tissue Culture:**
    - **Biopsy:** If previous tests are inconclusive, a biopsy of periprosthetic tissues may be performed to identify the pathogen. This is particularly important for diagnosing infections caused by fungi or slow-growing bacteria.
6. **Special Considerations for Fungi:**
    - In cases where fungi (such as *Candida* spp.) are detected, further evaluation and personalized antifungal treatment are essential. Fungal infections may be underdiagnosed if relying solely on standard bacterial cultures, and techniques like sonication or advanced molecular methods may be necessary for accurate identification.
7. **Differential Diagnosis:**
    - Before confirming the diagnosis of PJI, it is essential to rule out other possible causes of the symptoms.

Implementation in Practice: The algorithm should be adapted to the resources available in each medical facility. For example, not all centers have access to advanced tests such as alpha-defensin or genetic analyses. Continuous training of medical staff is essential to stay updated with new diagnostic techniques.

This algorithm provides a systematic framework for diagnosing periprosthetic joint infections, integrating both classical tests and modern technologies, and can be adjusted based on the specifics of each case and the availability of resources

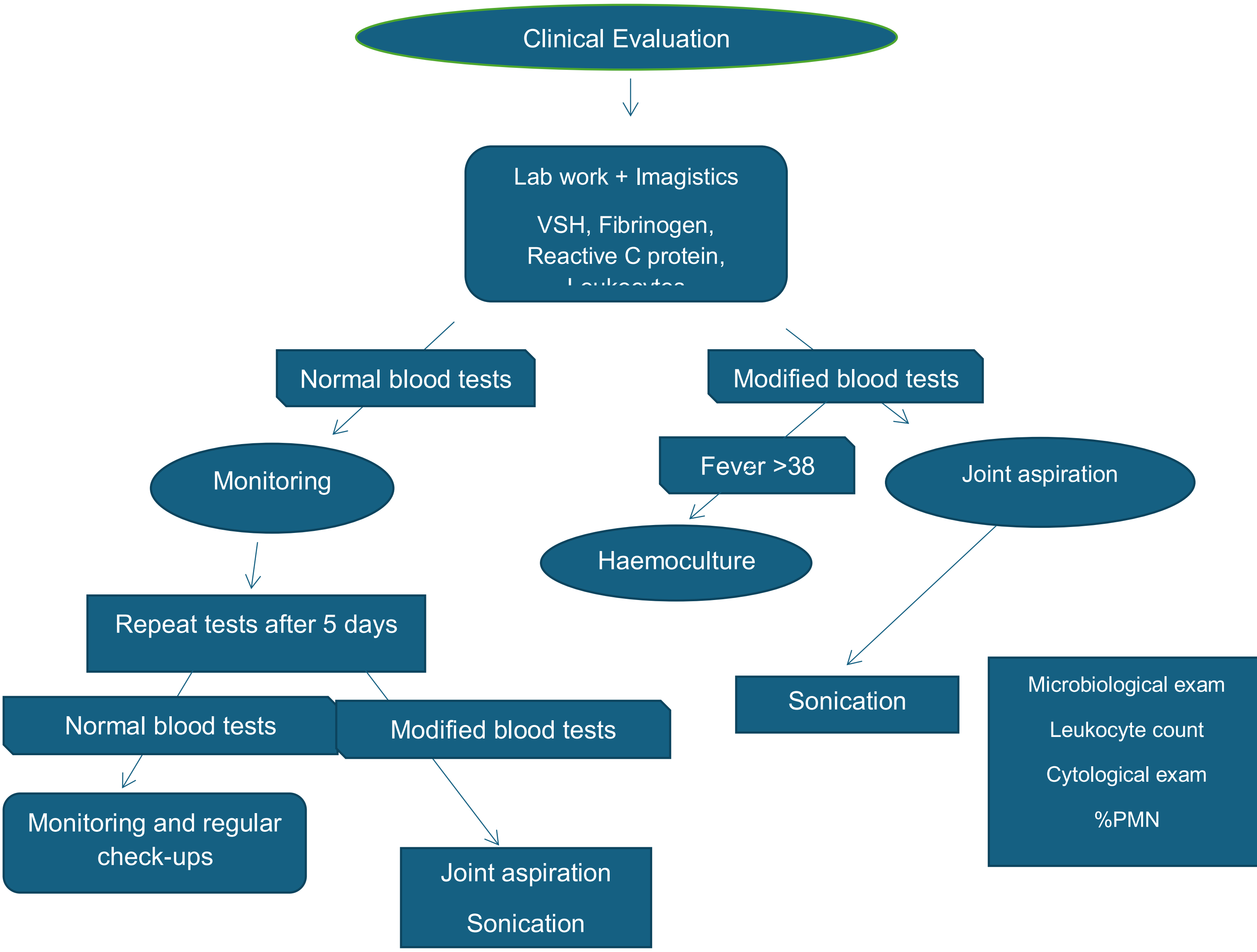

Figure 7.1 Diagnostic Algorithm for Periprosthetic Joint Infections (PJI)

7.7. Treatment Algorithm for Periprosthetic Joint Infections (PJI)

I propose the use of this therapeutic management guide for periprosthetic joint infections, adapted in accordance with the Guidelines for the Prevention and Control of AMR and HAIs from the National Institute of Infectious Diseases "Matei Balș." This guide is designed to provide physicians with a clear and well-defined set of therapeutic procedures, based on the latest evidence and best practices, to ensure effective and prompt treatment of periprosthetic joint infections.

General Principles of Judicious Antibiotic Use

1. **Targeted Therapy:** Utilizing protocols tailored to current bacterial resistance profiles is essential.
2. **Consultation with Infectious Disease Specialists:** In complex cases where standard protocols are not applicable, consulting a specialist is crucial.
3. **Antibiotic Stewardship Program:** Implementing such a program in hospitals can significantly impact antibiotic prescriptions, reducing microbial resistance and associated costs.

Perioperative Antibiotic Prophylaxis

Postoperative infections are among the most common healthcare-associated infections. The goal of perioperative antibiotic prophylaxis is to prevent the proliferation of colonizing germs that could cause infections following surgical interventions. Indications for antibiotic prophylaxis include:

1. **Indication for Antibiotic Prophylaxis:** Prophylaxis is recommended for surgeries classified as class II-III (clean-contaminated and contaminated surgery).
2. **Clean Operations:** These do not require prophylaxis, and infected operations require treatment, not prophylaxis.

Parameters for Perioperative Antibiotic Prophylaxis:

- **Timing of Administration:** The antibiotic should be administered 30-60 minutes before surgery to achieve effective serum and tissue concentrations.
- **Antibiotic Dose:** The standard therapeutic dose should be administered, adjusted for patients with higher body weight.
- **Duration of Administration:** Generally, prophylaxis should only be administered as long as necessary to ensure effective concentration during the surgery.

- **Antibiotics Used:** Narrow-spectrum antibiotics, such as first or second-generation cephalosporins, are preferred to reduce the risk of selecting resistant strains and Clostridioides difficile infections.

Treatment of Periprosthetic Joint Infections (PJI)

Diagnosis of Periprosthetic Infections: Early identification of the infection is crucial for successful treatment. Thorough clinical and paraclinical evaluations are recommended, including bacterial cultures and imaging tests.

Therapeutic Strategies:

1. **Debridement and Prosthesis Retention:** This is an option for acute infections or infections caused by antibiotic-sensitive bacteria.
2. **Two-Stage Prosthesis Removal and Replacement:** This protocol is recommended in cases of chronic infections or when resistant bacteria are involved.
3. **Antibiotic Therapy:** The choice of antibiotics is based on bacterial sensitivity. Intravenous therapy is initially recommended, followed by prolonged oral treatment.
4. **Monitoring and Post-Treatment Follow-Up:** Patients should be closely monitored for signs of infection recurrence, and antibiotic therapy can be adjusted based on clinical evolution and culture results.

- **Perioperative Antibiotic Prophylaxis**
  - **Preferred Antibiotic:** Cefuroxime 1.5 g intravenously (IV), administered 30-60 minutes before incision. For patients weighing >120 kg: 3 g IV.
  - **Alternatives for Patients with Beta-Lactam Allergy:** Clindamycin adult dose: 600-900 mg IV, administered 30-60 minutes before incision.
  - **Vancomycin adult dose:** 1 g IV (1.5 g for patients >80 kg) administered in a slow infusion (at least 60 minutes).
  - **Re-administration of Antibiotics:** Cefazolin: If the surgical procedure lasts more than 4 hours or there is significant blood loss (>1500 ml), an additional dose of cefazolin can be administered.
- **Treatment of Periprosthetic Joint Infections**
  - **A. Acute Infections (within the first 3-4 weeks postoperatively):**
    - **Surgical Debridement + Lavage + Implant Retention + Antibiotic Therapy (DAIR):** Intravenous antibiotic therapy: Cefuroxime 1.5 g IV

every 12 hours or Clindamycin: 600-900 mg IV every 8 hours + Vancomycin: 1 g every 12 hours (dose adjusted for renal function).
  - **Treatment Duration:** 2-6 weeks, depending on infection severity and response to treatment, followed by oral antibiotic therapy up to 6-12 weeks postoperatively.
  - **Replacement of Mobile Components** (head and insert for hip prosthesis and insert in case of knee prosthesis).
  - **DAIR Indication:** Known pathogen and well-fixed prosthesis.
- **B. Acute Infections (between 4 and 12 weeks postoperatively):**
  - **One-Stage Revision + IV Antibiotic Therapy:** Cefuroxime 1.5 g IV every 12 hours or Clindamycin: 600-900 mg IV every 8 hours + Vancomycin: 1 g every 12 hours or Linezolid 600 mg every 12 hours.
  - **One-Stage Revision Indication:** Patient in good general condition, known pathogen, sensitive to most antibiotics, without soft tissue extension.
  - **OR Two-Stage Revision:**
    - **Stage 1:** Removal of the infected prosthesis + surgical debridement + Lavage + Antibiotic Spacer + IV Antibiotic Therapy for 3-6 weeks: Cefuroxime 1.5 g IV every 12 hours or Clindamycin: 600-900 mg IV every 8 hours + Vancomycin: 1 g every 12 hours or Linezolid 600 mg every 12 hours, followed by oral antibiotic therapy for 8-12 weeks.
    - **Stage 2:** Reimplantation of the prosthesis after 8-12 weeks of IV antibiotic therapy, in the absence of clinical signs of infection, followed by continued antibiotic therapy for an additional 6 weeks.
  - **Two-Stage Revision Indication:** When the pathogen is unknown, there are bone defects or fractures, and when there is involvement of adjacent soft tissues.
- **C. Chronic Infections (>3 months postoperatively) or Infections Associated with Resistant Bacteria:**
  - **Two-Stage Prosthesis Removal and Replacement:**
    - **Stage 1:** Removal of the infected prosthesis, surgical debridement, and placement of an antibiotic spacer. Intensive IV Antibiotic Therapy for 3-6 weeks: Vancomycin 1 g IV every 12 hours + an antibiotic active against Gram-negative bacteria: Cefuroxime 1.5 g

IV every 12 hours or Meropenem: 1 g IV every 8 hours (in severe cases or with multi-resistant bacteria involvement), followed by oral antibiotic therapy for 8-12 weeks.

- **Stage 2:** Reimplantation of the prosthesis after 8-12 weeks of antibiotic treatment, in the absence of clinical signs of infection, followed by post-implantation antibiotic therapy.

- **D. Maintenance Therapy:**
    - **Long-Term Oral Therapy:** (after completing IV treatment) Rifampin: 300-450 mg orally every 12 hours + an oral antibiotic active against staphylococci: Cotrimoxazole: 960 mg orally every 12 hours or Doxycycline: 100 mg orally every 12 hours.

Monitoring and Adjusting Treatment

- **Monitoring Renal Function and Serum Levels:** Monitoring renal function and serum vancomycin/glycopeptide levels for dose adjustment. Assessing clinical response to treatment using inflammatory parameters (CRP, ESR, Fibrinogen) and possibly repeating joint aspiration.

Chapter 8. Conclusions and Personal Contributions

8.1 Conclusions

This doctoral thesis evaluated the role of sonication in the diagnosis and treatment of periprosthetic joint infections (PJI) compared to traditional and modern methods. The study demonstrated that sonication is an innovative and effective technique, with a sensitivity of 85% and a specificity of 97.5%, superior to traditional bacterial culture. Sonication disrupts bacterial biofilms, allowing for more precise identification of pathogens, including anaerobic bacteria and hard-to-detect strains. Compared to PET-CT, sonication offers similar diagnostic performance but is more economically accessible.

The thesis highlighted the importance of sonication in improving the therapeutic management of PJIs, facilitating the prompt initiation of appropriate antibiotic treatment and reducing the risk of recurrence. Combining sonication with other diagnostic methods, such as PCR and MRI, optimizes diagnostic accuracy and allows for a multimodal approach, essential for personalized treatment.

The economic and accessibility challenges associated with sonication and PET-CT emphasize the need for financing strategies and subsidies, as well as investments in equipment and medical staff training, to expand access to these advanced technologies, particularly in rural areas.

The thesis recommends integrating sonication into clinical guidelines for the diagnosis of periprosthetic infections, in combination with traditional methods, to ensure a complete and accurate diagnosis. Promoting ongoing research and adopting these advanced technologies can significantly improve patient outcomes and optimize therapeutic strategies.

8.2 Personal Contributions

Our study demonstrated that sonication represents a superior method for diagnosing periprosthetic joint infections (PJI) compared to traditional culture methods. The following points summarize the key findings and recommendations:

1. **Efficacy of Sonication**: Sonication has shown superior efficacy in diagnosing PJIs by detecting a broader range of microorganisms, including anaerobes and difficult-to-culture strains.
2. **Impact of Rapid Diagnosis**: Precise and rapid diagnosis through sonication allows for the prompt initiation of appropriate antibiotic treatment, reducing the need for empirical therapies and decreasing the risk of infection recurrence.
3. **Contribution to Infection Management**: Sonication provides more accurate data for monitoring inflammation and adjusting treatment, thereby improving the management of PJIs and clinical outcomes for patients.
4. **Combined Efficacy of Methods**: Using sonication in combination with surgical and rehabilitation treatments can significantly improve patient prognosis and quality of life.
5. **Sensitivity and Specificity of Sonication**: Implant sonication has demonstrated remarkable performance, with a sensitivity of 85% and a specificity of 97.5%.
6. **Recommendations for Future Research**: Future research should focus on randomized, multicenter clinical trials to evaluate the performance of diagnostic methods and develop new technologies for managing PJIs.
7. **Evaluation of Microbiological Methods**: Bacterial culture and PCR offer variable sensitivity and specificity, being essential in identifying pathogens in PJIs.
8. **Efficiency of Imaging Methods**: MRI and bone scintigraphy provide detailed images of soft tissues and bone structures, useful for detecting complications of PJIs.
9. **Use of Sonication in Rural Settings**: For patients from rural areas, sonication has shown a greater diversity of microorganisms, including Candida, compared to traditional methods.

10. **Detection of Microorganisms**: Sonication proved more effective in identifying a wider range of microorganisms, including Gram-negative bacteria and fungi, compared to joint aspiration.
11. **Limitations of PCR Method**: PCR has shown a sensitivity of 85% and specificity of 90%, being rapid and precise for detecting bacterial DNA, but it requires specialized equipment and expertise.
12. **Importance of a Multimodal Approach**: Diagnosing PJIs requires a multimodal approach, combining microbiological and imaging methods to obtain a complete and accurate picture of the infection.
13. **Percentage of Germ-Free Samples**: In patients diagnosed by joint aspiration, the percentage of germ-free samples was lower with sonication, suggesting higher sensitivity of the sonication method.
14. **Efficiency of Sonication by Age Group**: Sonication proved more effective in detecting infections in certain age groups, especially in patients aged 75 to 81 years.
15. **Integration of Sonication into Clinical Practice**: Sonication should be widely adopted in clinical practice to ensure more efficient diagnosis and management of PJIs, improving patient prognosis and reducing the costs associated with prolonged treatments and infectious complications.
16. **Use of Bacteriophages in PJI Treatment**: The use of bacteriophages in treating PJIs represents an innovative and promising approach in modern medicine, offering an effective alternative to conventional antibiotic therapies, especially in the context of increasing antimicrobial resistance. Due to their high specificity and ability to destroy resistant bacteria, bacteriophages can significantly contribute to managing complex infections associated with medical implants. However, to fully realize the potential of this therapy, further research and the establishment of clear standards for the safe and effective use of bacteriophages in clinical practice are necessary.
17. **Syndromic Testing**: Syndromic testing represents a significant advancement in the rapid and precise diagnosis of infections, allowing for the simultaneous identification of multiple pathogens directly from clinical samples. This method offers clear advantages by reducing diagnostic time and optimizing treatment, ensuring a more personalized and effective approach to infection management. However, widespread implementation of syndromic testing requires investments in

technology and training of medical personnel, making its integration into clinical workflows essential to significantly improve patient outcomes.

18. **Percentage of Germ-Free Samples**: A lower percentage of germ-free samples was observed in patients diagnosed through sonication compared to joint aspiration, indicating a higher sensitivity of the sonication method.
19. **Efficiency of Sonication Based on Age**: Sonication has proven to be more effective in detecting infections in certain age groups, particularly in patients aged 75 to 81 years.
20. **Integration of Sonication in Clinical Practice**: The widespread adoption of sonication in clinical practice is recommended to ensure more efficient diagnosis and management of PJIs, leading to improved patient prognosis and reduced costs associated with prolonged treatments and infectious complications.
21. **Bacteriophage Therapy for PJI**: The use of bacteriophages in treating PJIs is a promising alternative to traditional antibiotic therapies, particularly in the context of rising antimicrobial resistance. The potential of bacteriophage therapy must be further explored through continued research and the establishment of clear treatment protocols.
22. **Syndromic Testing**: Syndromic testing represents a major advancement in diagnosing infections by allowing for the simultaneous detection of multiple pathogens from a single clinical sample, reducing diagnostic time and improving treatment strategies.

In conclusion, this dissertation contributes valuable insights into improving the diagnosis of PJIs and emphasizes the importance of continued innovation and research in this critical field. The integration of sonication into routine clinical practice marks a significant advancement, providing more accurate and rapid diagnosis, better treatment management, and ultimately, a higher quality of life for patients. Continued research and the development of new technologies will support ongoing progress in orthopedics, offering substantial benefits to public health and individual patient care.

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