DOI:10.14753/SE.2024.2998

SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

2998.

KRIZSÁN GERGELY

A támasztó és mozgató szervrendszer működésének fiziológiája

című program

Programvezető: Dr. Szőke György, egyetemi tanár

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DOI:10.14753/SE.2024.2998

THE EFFECT OF RIFAMPICIN-RESISTANCE AND OTHER RISK FACTORS ON THE RECOVERY RATE IN PATIENTS WITH PROSTHETIC JOINT INFECTION

PhD thesis

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Budapest
2024

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List of Abbreviations

AAOS	American Academy of Orthopaedic Surgeons
ALBC	antibiotic-loaded bone cement
ASA	American Society of Anesthesiologists
BC	blood culture
BMI	body mass index
BSI	bloodstream infection
CDI	*Clostridioides difficile* infection
CFU	colony forming unit
CNS	coagulase-negative Staphylococcus sp(p).
COPD	chronic obstructive pulmonary disease
CRF	chronic renal failure
CRP	C-reactive protein
DAIR	Debridement, Antibiotics and Implant Retention
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DTT	difficult to treat
ECM	extracellular matrix
EPS	exopolysaccharide
ESBL	extended spectrum β-lactamase
ESR	erythrocyte sedimentation rate
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HMWP	high molecular weight polyethylene
HT	hypertension
ICD	implantable cardioverter defibrillator
IDSA	Infectious Diseases Society of America
IQR	interquartile range
IVDU	intravenous drug user
LFT	liver function test
LRE	linezolid-resistant Enterococcus sp(p).

MDRO	multidrug resistant organism
MIC	minimal inhibitory concentration
MRCNS	methicillin-resistant coagulase-negative Staphylococcus sp(p).
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
MRSE	methicillin-resistant *Staphylococcus epidermidis*
MSIS	MusculoSkeletal Infection Society
NGS	next-generation sequencing
OPG	orthopantomography
PCR	polymerase chain reaction
PJI	prosthetic joint infections
PMC	pseudomembraneous colitis
PMMA	polymethyl-methacrylate
PMN	polymorphonuclear cells
RA	rheumatoid arthritis
SD	standard deviation
TB	tuberculosis
TEE	transesophageal echocardiography
THA	total hip arthroplasty
TKA	total knee arthroplasty
UPC	unexpected positive culture
USA	United States of America
USD	United States Dollar
UTI	urinary tract infection
vif	variance inflation factor
VRE	vancomycin-resistant Enterococcus sp(p).
WCC	white cell count
WHO	World Health Organization

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1. Introduction

The number of total joint replacements shows an increasing trend due to the higher incidence of degenerative joint diseases (primary arthrosis) in the ageing population worldwide. Besides age, obesity (BMI >30 kg/m^2) and physical inactivity are also risk factors. However, patients with prosthetic implant due to secondary arthrosis as a result of trauma also represent a significant group. Prosthetic joint infections (PJIs) have become significant medical challenge over the last decades as a result of increasing number of arthroplasties and the higher age of patients involed, among other reasons [1]. PJIs are more likely to develop after revision than after primary endoprosthetic implantation [2].

The implantation of prosthetic devices is a high risk surgical procedure with possible complications including early, low-grade and haematogenic infections [3]. Prosthetic joint infections (also called periprosthetic infections) represent significant burden for both patients and health care providers resulting in further surgical interventions and long-term antibiotic treatment as well as longer hospitalisation and higher costs [4]. It is more difficult to effectively treat PJIs when mature biofilm has already been formed on the surface of the implant [5]. Treatment of prosthetic joint infections has therefore become an increasingly important area in orthopaedics.

The prevention of PJIs is based on appropriate surgical procedure and adequate antibiotic prophylaxis before (and in certain cases during) surgery. Therapeutic strategies are based on the combination of surgery and adequate antibiotic therapy. Appropriate surgical care and targeted antibiotic combination are essential for patients with PJI. Rifampicin is a regular component of the antibiotic combination due its excellent activity against biofilms, however, it can only be used in selected cases and there is a high risk of resistance development. Resistance towards rifampicin can be natural (intrinsic, primary) or acquired (secondary) and it represents a significant challenge resulting in longer hospitalisation, increased costs as well as higher mortality [6].

1.1. The short history of prosthetic joint infections

The first prosthetic implantations have been performed in the 1970s by Sir John Charnley [7]. The basic concept of low friction arthroplasty is a low diameter prosthesis head facing the plastic acetabulum. Both components are fixed in the correct position with bone cement. The acetabulum was initially made of teflon, however, degradation of the prosthetic material after about 3 years induced significant reaction in surrounding tissues. However, the application of high molecular weight polyethylene (HMWP) offered acceptable outcomes [8].

Beyond the initial mechanical issues another challenging complication appeared: deep-seated infections around the prosthesis. Prosthetic joint infections were recognised shortly after modern implant devices were introduced. After the first 100 hip prosthetic implants as high as 10% of the patients had to face this serious complication. Sir John Charnley suggested that if the ratio cannot be reduced to less than 5%, prosthetic joint implantations should only be considered in elderly patients with significant comorbidities and in general osteotomy should be preferred given the lower risk of infections [8]. On the other hand, the incidence of PJIs has dropped with more advanced technologies including laminar air-flow, aseptic techniques, antibiotic prophylaxis, shorter duration of surgery and antibiotic-containing bone cements, developed by H. W. Buchholz to act locally as prophylaxis [9].

However, the clinical significance of PJIs is still crucial due to the increasing number of primary and revision surgical procedures. Between 2001 and 2009, 2.0-2.4% of primary hip and knee joints had to undergo revision due to early or low-grade infection [10]. The number of hip joint revisions are expected to increase by 137% and knee revisions by 601% between 2005 and 2030 in the USA. The rising incidence of PJIs represents a significant burden to health care systems due to higher expenses and increased drug consumption as well as prolonged hospitalisation and delayed mobilisation of patients [11]. The annual cost of PJIs are estimated to be 1.62 billion USD from 2020 in the United States [1, 7].

1.2. Characterisation and significance of biofilms

Biofilm formation on the surface of the prosthetic material is a significant challenge in the treatment of prosthetic joint infections. Recognition of the clinical significance of biofilms substantially helped understand the patomechanism of PJIs [12, 13]. Biofilms consist of microorganism cells (20-30%) and extracellular matrix (ECM) or glycocalyx produced by bacteria (70-80%). The ECM contains DNA, glycoproteins, glycolipids and exopolysaccharides (EPS). In healthy individuals biofilm production is under control due to the activity of the immune system [14]. However, no appropriate immunological protection can be provided when prosthetic material is present. In the lack of local immunity, even a low number of bacteria (a few hundred cells) are able to adhere and produce glycocalyx providing favourable surface for further adhesion and proliferation [15].

Bacteria have two metabolic forms: planctonic (free) and sessile (fixed) [16]. The development of biofilm begins with the reversible adhesion of planctonic cells initially binding to the surface of the implant with weak electrostatic and *van der Waals* forces followed by irreversible cell adhesion interactions (eg. with pili) [17]. A structure consisting of multiple layers of bacterial cells is called the early biofilm. Production of extracellular matrix is the next step with simultaneous proliferation of bacterial cells resulting in mature biofilm. An equilibrium is reached where the number of bacterial cells within the biofilm is increased by proliferation and reduced by the release of planctonic cells called dispersion. On the top of direct spread, the latter is another way of extension of biofilm on the prosthetic surface (Figure 1.).

There are four main reasons of biofilm production according to Jefferson *et al*. [18]:

1. Protection against external effects (eg. pH-alterations, antimicrobial agents, components of the immune system).
2. Facilitate anchoring to suitable areas resulting in colonisation of the surface (natural or artificial) and provide a protected niche for bacteria.
3. Share metabolic burdens (eg. co-metabolism of xenobiotics) and stimulate horizontal gene transfer resulting in the spread of antimicrobial resistance.
4. Primary appearance for certain species that can only live in planctonic form under unfavourable circumstances.

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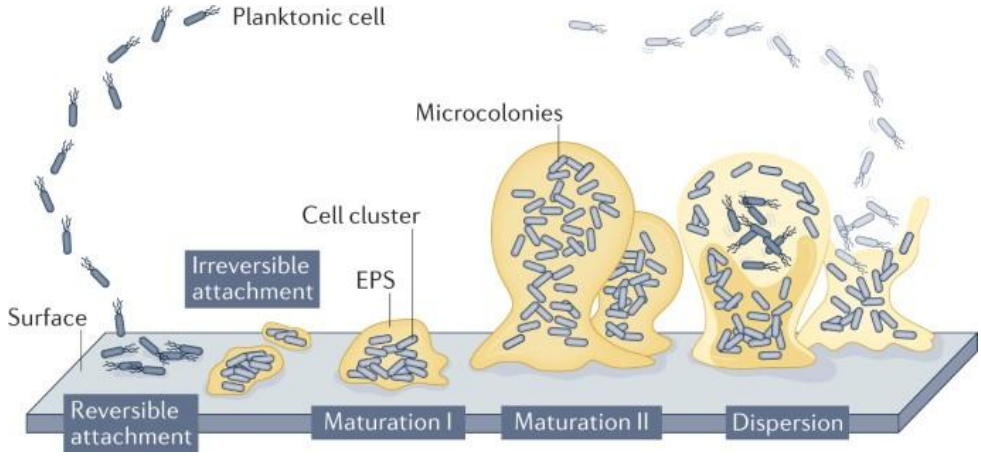

Figure 1. The life cycle of biofilm [19]

There are several clinical challenges in relation to biofilms. First of all, bacteria embedded in biofilms are substantially less accessible for antibiotics, hence it is more difficult to achieve therapeutic concentrations of antibiotics resulting in higher risk of treatment failure [20]. Secondly, bacteria are highly protected from the immune system and therefore no appropriate response can develop to control the infection [21]. Not even the adhesion and colonisation itself can be prevented under these conditions. Third, the adhesion of bacteria to the surface of the prosthesis makes the eradication of bacteria more difficult from a mechanical point of view in biofilm-associated infections [22]. It is also important to note that biofilms can also be formed on the surface of native tissues pointing out the importance of thorough debridement as part of the surgical procedures. It normally takes 4-6 weeks to develop mature biofilm on the surface of prosthetic materials. Since one of the fundamental aims of surgical treatment is to eradicate biofilm, it is essential to choose the appropriate procedure according to the maturity stage of the biofilm [23, 24].

1.3. Prosthetic joint infections (PJI)

Without effective local immune response a low amount of bacterial cells (less than 1000 cells) are able to proliferate and develop infection. Diagnostics of periprosthetic infections can be challenging in many cases. There were no available harmonised guidelines previously to establish evidence-based diagnostic and therapeutic protocols. Various classifications are available nowadays including Tsukayama-classification

differentiating four types of prosthetic joint infections [25]. However, more recently the following three types are accepted: early postoperative (0 – 4-6 weeks), low-grade (6 weeks to 2 years) and late haematogenic infections (after 2 years).

In an other approach the three main routes of an implant becoming infected are intraoperative, late haematogenic and direct spread from the inflamed tissue around the prosthesis which may be due to various conditions including wound infection and osteomyelitis [26]. Intraoperative and postoperative infections are the most frequent types [17].

1.3.1. Early postoperative infection

Prosthetic joint infections developing within 4-6 weeks [24] or 30 days [27] after prosthesis implantation are classified as early infections. Clinical symptoms include erythema, swelling and pain around the infected joint. This type also comprises cases with impaired early wound healing characterised by dehiscence, discharge, necrosis of wound edges as well as fever, shivering and being generally unwell. As long as the biofilm is not mature, there is a chance to save the implant [28].

There are different manifestations of early postoperative infections [29]. Ongoing leak does not necessarily represent infection, however, it can act as a port of entry for pathogens leading to secondary infections potentially spreading to the prosthesis within deep tissues in case of suture insufficiency [30, 31]. It is being investigated at what stage discharging wounds should be explored [32]. According to the guidance of International Consensus Meeting Philadelphia, exploration and surgical treatment of discharging wounds is recommended after 5-7 days [30]. Another characteristic manifestation of early infection occurs without wound discharge, however, clinical symptoms of joint infection can develop as early as 7-10 days after surgery.

This type of prosthetic joint infection is predominantly caused by *Staphylococcus aureus* and β-haemolytic Streptococcus spp. [33, 34]. Symptoms develop suddenly without prodromal sings resulting in decreased mobility. Pathogens spread from a different source of infection and reach the implant with the bloodstream (haematogenous PJI). Most frequent sources are skin and soft tissue infections, urinary tract infections, respiratory and gastrointestinal foci [35, 36] (Figure 2.). Biofilm is not

mature in these cases either making it possible to save the prosthesis [28]. Isolates with higher level of resistance (eg. MRSA) represent a further risk factor resulting in more difficult eradication of the infection [37].

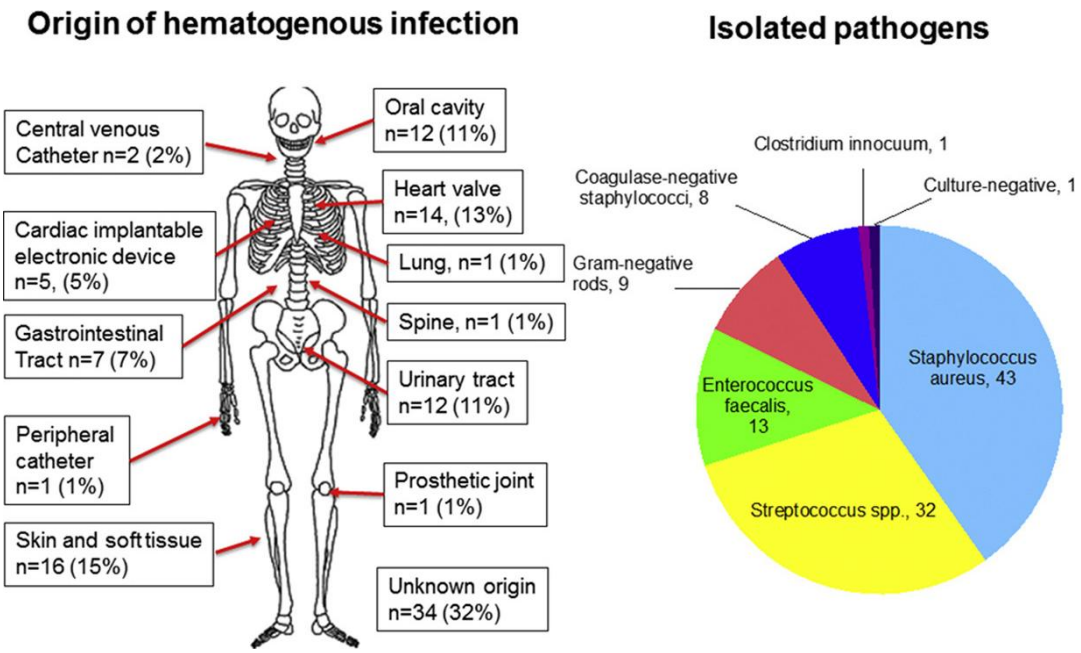

Figure 2. Origin and characteristics of haematogenous periprosthetic joint infection [38]

1.3.2. Low-grade infection

Infections developing between 6 weeks and 2 years postoperatively are classified as low-grade infections. It is very important to point out that most of the periprosthetic infections are recognised at this stage [39]. Low-grade infections are generally caused by low-virulence bacteria, eg. *Staphylococcus epidermidis*, *Cutibacterium acnes* and other microorganisms from the skin flora as well as Acinetobacter spp. This type of infection is characterised by decreasing functions of the joint, painful limited movements and possible fistula formation. Clinical symptoms include fluctuating pain and intermittant swelling. Biofilms are mature at this stage and even certain components of the prosthetic material may loosen by this time. Therefore it is considered unavoidable to completely remove the implant [24, 40]. However, a study found that DAIR procedure may still be successful in selected cases [41].

1.3.3. Late haematogenic infection

This type of PJI can develop any time more than 2 years after surgery. Although the prosthesis is functioning well and the patient has no obvious prodromal symptoms, the infected joint rapidly becomes painful and shows limited movement functions [42]. The pathogenic microorganism can reach the prosthetic material from a distinct source of infection via bloodstream and adheres to artificial surface. If the patient has more prosthetic devices *in situ*, they all may be involved. The most frequent sources are skin and soft-tissue infections, however urinary tract, respiratory tract and gastrointestinal or oropharyngeal infections can also act as the original focus [35, 36], similarly to acute cases. Predisposing factors include immunosuppression, diabetes mellitus and chronic renal failure. The most important characteristics of different types of prosthetic joint infections are summarised in Figure 3.

1.4. Microbiological background

Prosthetic joint infections can be caused by a huge number of different microorganisms, however, certain species are far more prevalent, eg. *S. aureus* and β-haemolytic Streptococcus spp. As most of the PJIs are recognised in the low-grade stage, microorganisms are mostly present in mature biofilm, making their identification with conventional microbiological methods more difficult in such cases.

	Time since surgery		
	0 to 2 months	3 to 24 months	Any time
Type of infection	Early infection	Delayed (low-grade) infection	Late infection
Route of infection	Perioperative	Perioperative	Hematogenous (focus usually on lungs, skin, urinary tract, dental) or continuous spreading from elsewhere
Clinical symptoms	Local reddening, overheating, fever, pain, wound dehiscence, secretion	Persistent or new-onset pain, loosening, fistula formation	Acute or subacute
Most common pathogens	Staphylococcus aureus, streptococci, enterococci	Coagulase-negative staphylococci, Propioni-bacterium acnes	Staphylococcus aureus, Escherichia coli, streptococci

Figure 3. Classification of periprosthetic infections [43]

In order to identify the causative agent, mature biofilm has to be detached from the surface of the implant and degraded in the next step. There are two main methods to achieve this on explanted prosthetic material: physical (for instance: sonication) and chemical (for instance: dithiothreitol). Sonication uses low frequency ultrasound to obtain microorganism cells from the biofilm [44]. This method significantly increases sensitivity, however, there is a high risk of contamination and yielding mixed cultures. It may prove difficult to accurately assess the clinical significance of the isolate(s). Dithiotreitol leads to chemical disassembly of the biofilm followed by the appearance of microorganisms in the planctonic phase becoming accessible for microbiological culture [45]. The risk of contamination is lower with dithiotreitol and the sensitivity is similar to that of sonication, however, this method is limited by its availability.

Prosthetic joint infections are mostly but not exclusively caused by various bacteria. Gram-positive bacteria include Staphylococcus spp., Streptococcus spp., Enterococcus spp., Corynebacterium spp. and Cutibacterium spp. [46]. A study found that coagulase-negative Staphylococcus spp. are the predominant causative agents of PJIs (30-43% of the cases) followed by *S. aureus* (12-23%). In general, Staphylococcus spp. are the most prevalent causative agents of PJIs followed by Streptococcus spp., primarily β-haemolytic species such as *S. pyogenes*, *S. agalactiae* and *S. dysgalactiae*. Of note, streptococcal infections most frequently develop on the basis of haematogenic infections. In another study 25% of the specimens yielded Streptococococcus spp. and blood cultures were also positive in 22% of the cases. Moreover, co-infection was identified in 22% of the patients with PJI, predominantly involving *S. aureus* [47]. Gram-negative bacteria include members of the *Enterobacterales* order („coliforms”), eg. *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Proteus mirabilis*, Citrobacter spp. and *Morganella morganii*. The other important group is called nonfermenters (obligate aerobic bacteria) such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* [48].

Rare isolates include Brucella spp. and yeasts, mostly Candida spp. [49]. However, no pathogen was identified in 28.2% of the cases by culture methods [50]. On the other hand, unexpected positive culture (UPC) results can be obtained during aseptic revision. The clinical significance of these findings are yet to be fully understood [51]. Whilst

Staphylococcus spp. are the most frequent causative agents, further Gram-positive and Gram-negative bacteria as well as fungal pathogens can also be involved, potentially resulting in polymicrobial infections [48, 52].

Both Gram-positive and Gram-negative microorganisms have the ability to form biofilm on the surface of prosthetic materials, particularly Staphylococcus spp. and *P. aeruginosa,* associated with less favourable outcomes [23, 53]. Moreover, difficult to treat (DTT) microorganisms have another clinically important feature: high-level resistance to various classes of antibiotics. Such isolates may be found up to 58% of the cases. This increasing group comprises Staphylococcus spp. resistant to rifampicin and most of other oral agents, methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative Staphylococcus spp. (MRCNS), Enterococcus spp. resistant to aminopenicillins (eg. *E. faecium*) and especially to vancomycin (vancomycin-resistant Enterococcus spp., VRE) or even linezolid (linezolid-resistant Enterococcus spp., LRE). Resistant Gram-negative bacteria include ESBL- and derepressed AmpC-producing isolates. Multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates can also be involved. Another challenge is that in infections caused by bacteria with extended resistance, there may be no oral antibiotic option available for treatment resulting in prolonged hospitalisation, increased risk of complications and acquisition of healthcare-associated infections as well as higher costs and lower recovery rates [52].

1.5. The risk factors of prosthetic joint infections

The risk and course of prosthetic joint infections are determined by various factors including virulence of the microorganism, patient's comorbidities as well as interactions between bacteria and the host. The significantly increased risk of PJIs was emphasized due to the duration of surgery and selected clinical conditions such as diabetes mellitus, high ASA score, concomitant urinary tract infection and immunosuppression [54, 55]. Another study highlighted the significance of obesity, chronic ethanol excess, COPD, coagulopathies and malignant diseases as risk factors in the development of PJIs and prolonged post-surgical recovery [56]. The following conditions are currently considered as risk factors: previous surgery of the involved joint, poorly controlled

diabetes mellitus (HbA1c >7% or serum glucose >200 mg/dL), obesity (BMI >30 kg/m^2), malnutrition, chronic renal failure, hepatitis and cirrhosis, smoking, chronic ethanol excess, intravenous drug abuse (IVDU patients), prolonged hospital stay or rehabilitation, male sex, posttraumatic osteoarthrosis or inflammatory arthropathy in the affected joint as well as immunosuppression [57]. Intraarticular corticosteroid injection is considered a risk factor both before (given within 3 months) and after arthroplasty [58, 59].

1.6. Diagnostics of prosthetic joint infections

The diagnostics of PJIs is based on taking complete past medical history, thorough physical examination as well as biochemical, microbiological, radiological and histopathological investigations [60]. The causative agent can ideally be identified and antimicrobial sensitivities can be determined before the surgical procedure, however, these are not obligatory criteria. Clinical suspicion of PJI arises in case of ongoing pain since implantation or if the joint rapidly becomes painful after a symptom-free period. On the other hand, diagnosis is more challenging in low-grade infections and rather the probability of infection can be estimated in such cases. A detailed diagnostic algoritm of prosthetic joint infections is shown on Figure 4.

Different diagnostic guidelines have been established for PJIs, however, the currently accepted consensus has been developed on the basis of previously issued algorithms from AAOS [62], IDSA [27] and MSIS [63]. The protocols have been amended and harmonised on the Consensus Conference in Philadelphia which is now the primary guidance of diagnostics of PJIs [63, 64] (Figure 5.). Previously one major criterium or at least 4 minor criteria were required for the diagnosis of PJI according to MSIS guidance. Major criteria were fistula communicating with the prosthesis and detection of the same microorganism twice from the involved joint (tissue or joint fluid), whereas minor criteria included raised erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), raised white cell count (WCC) or increased leukocyte esterase activity in the synovial fluid, increased neutrophil/granulocyte ratio (PMN%) in the synovial fluid, macroscopically visible purulent discharge in the infected joint, pathogen cultured once and more than 5 neutrophil granulocytes on microscopic histology examination with

400x magnification. The most recent guideline in 2018 also includes major and minor criteria. Major criteria are the same as before and one major criterium confirms PJI, however, minor criteria have scores and they are now divided into preoperative and intraoperative group [65].

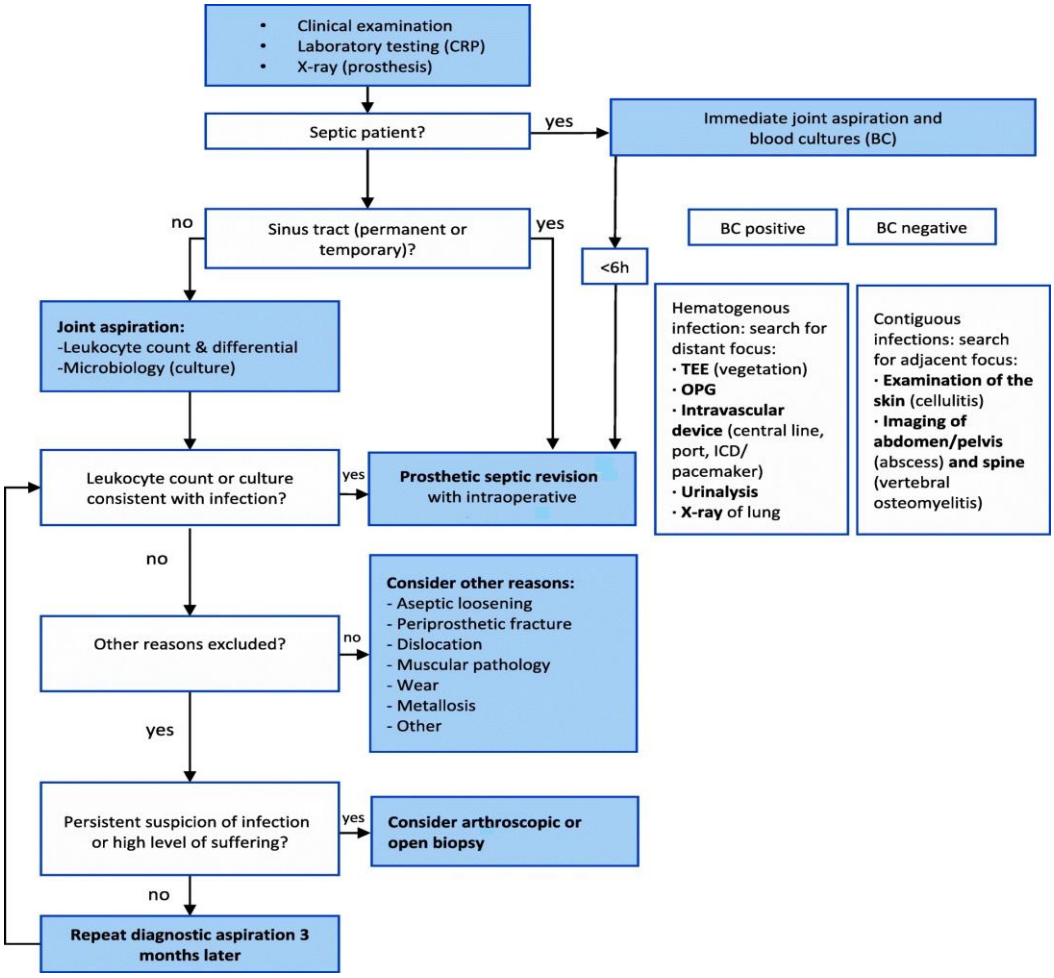

Figure 4. Diagnostic algoritm of prosthetic joint infections [61]

Another algorithm has been developed by the European Bone and Joint Infection Society [67] (Figure 6.).

Appropriate microbiological sampling procedure has a fundamental role in the diagnostics and treatment of PJIs. Specimens should be taken before the initiation of systemic antibiotic treatment, ie. antimicrobial treatment should be given after sampling and/or during surgery [68, 69]. The two main types of sampling are arthrocentesis (aspiration of joint fluid) and intraoperative tissue biopsy. When arthrocentesis is performed, skin disinfection is essential and the use of lidocain should be avoided.

Major criteria (at least one of the following)	Decision
Two positive cultures of the same organism	Infected
Sinus tract with evidence of communication to the joint or visualization of the prosthesis	

		Minor Criteria	Score	Decision
Preoperative Diagnosis	Serum	Elevated CRP *or* D-Dimer	2	≥6 Infected 2-5 Possibly Infected [a] 0-1 Not Infected
		Elevated ESR	1	
	Synovial	Elevated synovial *WBC count or LE*	3	
		Positive alpha-defensin	3	
		Elevated synovial PMN (%)	2	
		Elevated synovial CRP	1	

	Inconclusive pre-op score *or* dry tap [a]	Score	Decision
Intraoperative Diagnosis	Preoperative score	-	≥6 Infected 4-5 Inconclusive [b] ≤3 Not Infected
	Positive histology	3	
	Positive purulence	3	
	Single positive culture	2	

Figure 5. 2018 International Consensus Meeting criteria for the diagnosis of PJI [66]

	Infection Unlikely (all findings negative)	Infection Likely (two positive findings)	Infection Confirmed (any positive finding)
Clinical and blood workup			
Clinical features	Clear alternative reason for implant dysfunction (e.g. fracture, implant breakage, malposition, tumour)	1) Radiological signs of loosening within the first five years after implantation 2) Previous wound healing problems 3) History of recent fever or bacteraemia 4) Purulence around the prosthesis	Sinus tract with evidence of communication to the joint or visualization of the prosthesis
C-reactive protein		> 10 mg/l (1 mg/dl)	
Synovial fluid cytological analysis			
Leukocyte count (cells/µl)	≤ 1,500	> 1,500	>3,000
PMN (%)	≤ 65%	> 65%	> 80%
Synovial fluid biomarkers			
Alpha-defensin			Positive immunoassay or lateral-flow assay
Microbiology			
Aspiration fluid		Positive culture	
Intraoperative (fluid and tissue)	All cultures negative	Single positive culture	≥ two positive samples with the same microorganism
Sonication (CFU/ml)	No growth	> 1 CFU/ml of any organism	> 50 CFU/ml of any organism
Histology			
High-power field (400x magnification)	Negative	Presence of ≥ five neutrophils in a single HPF	Presence of ≥ five neutrophils in ≥ five HPF
			Presence of visible microorganisms
Others			
Nuclear imaging	Negative three-phase isotope bone scan	Positive WBC scintigraphy	

Figure 6. The EBJIS definition of periprosthetic joint infection [67]

Tissues can be taken from the synovial membrane and joint capsules (hip and knee), the periacetabular region (hip) and the peripatellar region (knee) [68, 70, 71]. It is crucial to avoid contamination when specimen is being taken: this can lead to the culture of more superficial or skin flora microorganisms that are clinically not relevant. They can also mask the true pathogen(s) and may result in excessive use of broad-spectrum antibiotics causing side effects and increasing antibiotic resistance. It is recommended to send at least 5 specimens for microbiological investigations from different parts of the site of infection including bone-prosthesis interface as well as 2 specimens for histology [72]. Radiological imaging techniques may be required for accurate sampling and repeated specimens may be necessary in certain cases. Sensitivity of this procedure is 65-95% [73].

Specimens should urgently be sent to the Microbiology Laboratory and processed immediately. Sterile container is appropriate, however, aspirated fluids can also be injected to sterile blood cultures [72]. Incubation of blood culture bottles in automated instruments provides enrichment (should bacterial count be minimal) and higher probability of identifying the causative agent. Similar pathway is followed in the diagnostics of bloodstream infections (BSI) as the bacterial concentration is usually very low (few cells in 1 mL blood) in such cases. Tissue specimens are also processed for direct culture and enrichment for the same reason. As previously described, physical and chemical methods (sonication and dithiotreitol) can remove bacterial cells from the biofilm of the implant surface and increase the sensitivity of diagnostics. Moreover, molecular methods, including 16S PCR and next-generation sequencing (NGS) offer further diagnostic opportunities [50].

1.7. Treatment of prosthetic joint infections

The therapeutic approach of PJIs should always be based on the combination of surgical and conservative management. The probability of biofilm formation and its stage should also be considered. Accurate diagnosis and correct microbiological identification and antimicrobial susceptibility testing of the causative agent are essential to provide optimal therapy [24, 74]. Infections should be classified on the basis of previously described score systems and clinical course to be able to optimise treatment and follow

up plan [24]. It is important to highlight that clinical manifestations of prosthetic joint infections include a wide range of symptoms from long-standing, mild infections resulting in gradual loosening of the implant up to fulminant cases leading to septic shock within a few hours. Therefore not only the classification but the urgency of the clinical picture will direct therapeutic approach. Delayed wound discharge without fever after primary surgery is not an urgent condition in most cases, however, if symptoms include fever, tachycardia, low blood pressure and the patient is generally unwell, imminent care is unavoidable.

1.7.1. Surgical treatment

1.7.1.1. Debridement, Antibiotics and Implant Retention (DAIR procedure)

Retention of prosthesis alongside with antibiotic treatment can be the first therapeutic choice in early postoperative (<6 weeks) and acute haematogenic infections (<3 weeks) (Figure 7.) as the infection in later stages can only be eradicated by complete exchange of the prosthesis [28]. All necrotic tissues are carefully debrided during the procedure including synovectomy and eradication of fistulae followed by extensive washout with 6-9 L of sterile or antiseptic fluid [75]. High-pressure fluid is generated called jet-lavage which is essential in DAIR procedure. The fluid can be physiological saline, Betadine, 0.02% chlorhexidine or a mixture of these compounds. It is recommended to remove or exchange modular components of the implant such as plastic insert of knee prosthesis and plastic acetabular insert and prosthesis head in hip joint [76]. The nonmobile elements of the prosthesis are left *in situ* during surgery. The aim of the procedure is to remove immature biofilm from the prosthetic surface, wash pathogens and their toxins out and save the implant. Arthroscopic debridement and irrigation shows less favourable outcomes compared to open surgery as mobile components cannot be exchanged and appropriate debdridement can not be provided [75, 77, 78]. The procedure is called DAIR standing for Debridement, Antibiotics and Implant Retention and it is followed by prolonged antibiotic treatment [79].

The aim of the procedure is to save the prosthesis by eradicating the infection and removing the biofilm. However, DAIR procedure cannot be used for the treatment of

late PJIs as compact and mature biofilm can only eradicated by the exchange of prosthesis [28]. There are different recommendations regarding antibiotic route and duration, however, all regimes include prolonged intravenous treatment followed by oral antibiotics.

The duration of intravenous antibiotic therapy ranges from 2 to 6 weeks and the total duration of treatment is 12 weeks in most of the cases, however, it can be as long as 24 weeks if knee joint is involved [62, 75, 81]. Moreover, treatment regime is also determined by the type and outcome of surgical procedure(s). Rifampicin has an essential role in the management of infections with biofilm formation [27]. However, the success rate of DAIR procedure shows declination as biofilm matures resulting in significantly lower efficacy beyond 30 days [28].

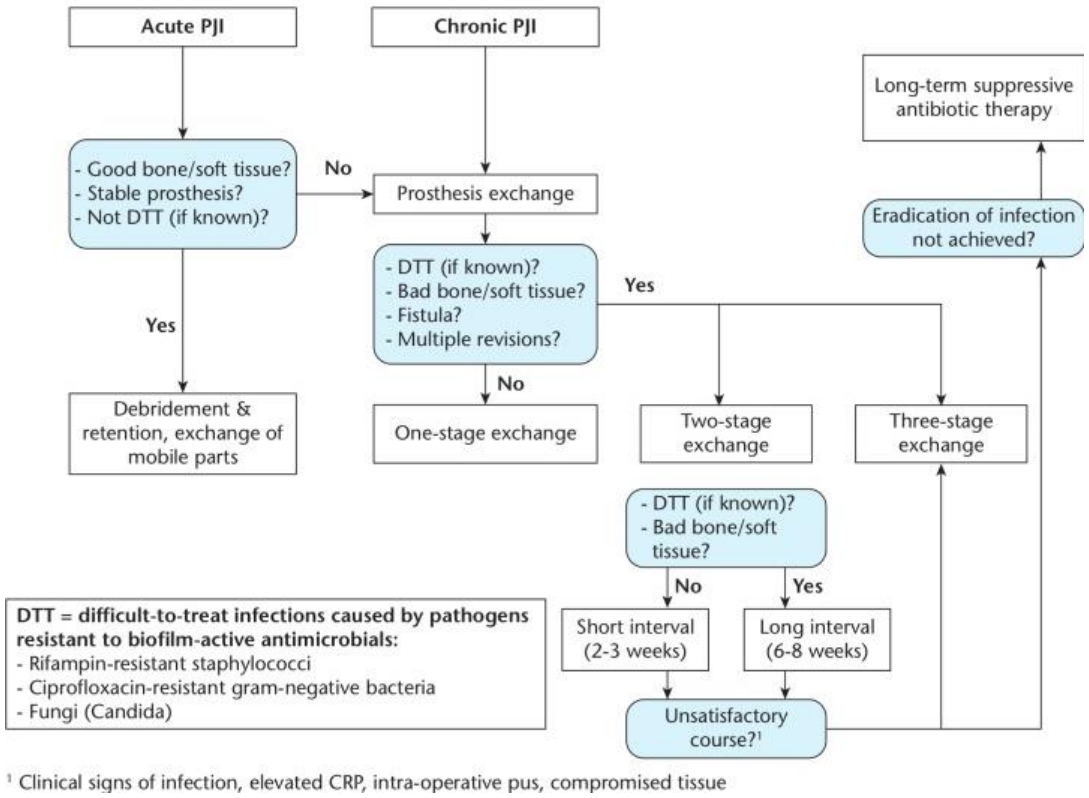

Figure 7. Treatment algorithm of prosthetic joint infections [80]

1.7.1.2. Revisions with exchange of the implant

Patients with long-standing symptoms are likely to have a prosthesis with mature biofilm, hence complete removal of the implant is unavoidable. There are different

types of such revisions: the most important ones are one-stage and two-stage procedures (Figure 8.). When one-stage revision is performed, the infected prosthesis is completely removed, followed by thorough debridement (including the „infect membrane") and a new prosthesis is implanted after extensive washout in the presence of local antibiotics [82]. In two-stage revisions the explantation of the old infected prosthesis is separated from the implantation of the new prosthesis in time. After the explantation, a spacer can be placed *in situ*, which is made of polymethyl-methacrylate (PMMA) loaded with antibiotics. The new prosthesis is implanted after the settling of local inflammatory reactions [62, 84].

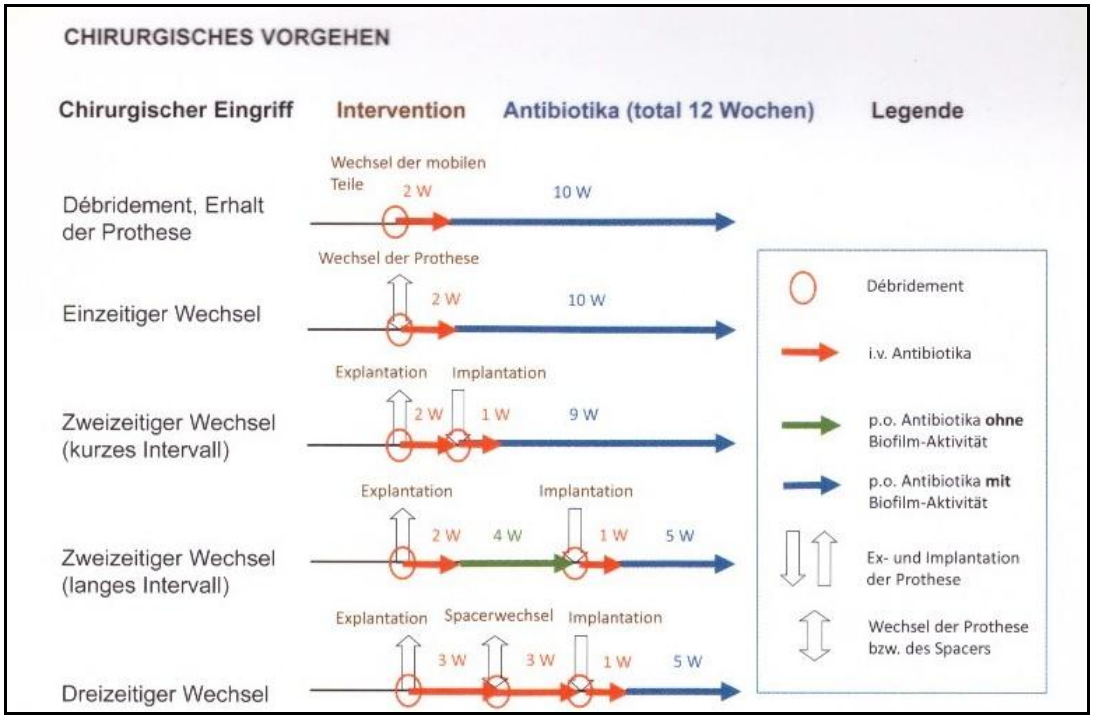

Figure 8. The types of surgical procedures in case of prosthetic joint infections [83]

Antibiotic therapy is required during the period between the two surgical procedures: at least two weeks of intravenous treatment followed by oral agents for a minimum duration of 4 weeks. There is no exact protocol for the timing of reimplantation, however, it is feasible to perform after 6-8 weeks if inflammatory parameters have returned to normal range [62, 75, 81]. It is advisable not to increase the interval significantly as soft tissue may shrink by time. Of note, in short interval two-stage revision there is only 2 weeks between the two surgical episodes, however, this procedure can only be followed when certain criteria are fulfilled.

Two-stage revision has been considered the „gold standard" procedure during the last decades. However, there is a recent trend to achieve conditions satisfactory to one-stage revision as higher morbidity, longer hospitalisation, prolonged immobilisation and increased healthcare expenses can be associated to two-stage revision whereas the success rate of one-stage revision has been shown to be non-inferior [85-87]. Reinfection rates and functional results are also more favourable according to patient responses when exploring quality of life [88]. On the other hand, one-stage revision has strict criteria: it requires preoperatively identified pathogen with available antibiogram, intact soft tissue, radical debridement, suitable bone tissue for the fixation of the implant, intraarticular antibiotic treatment achieving bactericidial concentrations and the possibility of postoperative intravenous antibiotic treatment [71, 89]. During the implantation cement with antibiotics can be used, otherwise sufficient local antibiotic concentration must be provided, eg. with antibiotic bone graft [90] or other carriers [91, 92]. Furthermore, three-stage revision procedure can also be mentioned. It includes a spacer exhange between implantation and explantation, the interval is usually 6 weeks long.

1.7.1.3. Resection arthroplasty

If PJI recurs despite of accomplishing different therapeutic procedures, it may be necessary to leave the joint without prosthetic material. In the hip the joint is left without fixation: the surgery is called Girdlestone-procedure. In the knee the joint is fixed, the term is arthrodesis and in other joints it is sine-sine plastics (or formation of pseudo-joint) [93]. In extremely rare cases when PJI cannot be controlled or circulation of the limb is poor and if there is no realistic chance to achieve resolution by keeping the limb, amputation above the infected joint may have to be considered.

1.7.2. Antimicrobial therapy

The complex therapeutic approach of prosthetic joint infections comprises antimicrobial treatment including empirical and targeted treatment at different stages. However, there are no standardised treatment guidelines available. The general principle is to provide at least 10-12 weeks of antimicrobial treatment [94]. Whenever surgery has to be

performed without identified pathogen and available antibiogram, debridement and irrigation must be followed by broad-spectrum antimicrobial cover in order to cover the most likely causative agents (eg. vancomycin, ceftriaxone). However, targeted treatment can only be initiated when microbiology results become available (de-escalation). Oral stepdown can be attempted at least 2 weeks after intravenous treatment has been started if inflammatory parameters are on a decreasing trend, the wound is dry and there is no clinical concern of infection. However, another approach suggests at least 6 weeks of intravenous treatment initially. It is recommended to choose antibiotic with excellent bone penetration, good bioavailability and bactericidal effect. Also, the agent should be suitable for long-term treatment in terms of side effects and drug interactions (Figure 9.). It is also recommended to monitor serum concentration of certain antibiotics during treatment. This test is limited to a few antibiotics in clinical practice. Normally trough levels are followed up whereas peak concentrations are usually not required. Low trough level indicates the risk of decreased therapeutic effect and also carries a risk of resistance development. High trough levels, on the other hand, may result in side effects or intolerance [95]. The most important examples are vancomycin, teicoplanin and gentamicin.

Protocols for the duration of antibiotic regimes show high variation. Continuous antibiotic cover is essential between the removal of the infected prosthesis and the reimplantation of the new device. Drug holiday, when antibiotic treatment is suspended for shorter periods (few days) is not recommended any more in this setting [80]. However, it may occur that the interval is so long that the patient is not going to be on antibiotics by the time when the new prosthesis is implanted. If re-implantation culture is negative, at least 6 weeks of antibiotics is required whereas 12 weeks is recommended when culture results are positive.

Even though surgical procedures are the most effective in the treatment of PJIs, they may not be feasible in certain cases. As a result, long-term, frequently life-long suppression antibiotic treatment becomes necessary which can also result in satisfactory outcomes and acceptable quality of life [97]. This usually happens with elderly patients with multiple comorbidities if further surgical procedures are contra-indicated or if there is a risk of further loss of bone tissues or even amputation of the limb or if the patient does not give consent. It may also be considered if the prosthesis is stable and the pain

is mild. The therapeutic aim in such cases is not the eradication of the pathogen but to diminish symptoms and complaints and keep the process in remission.

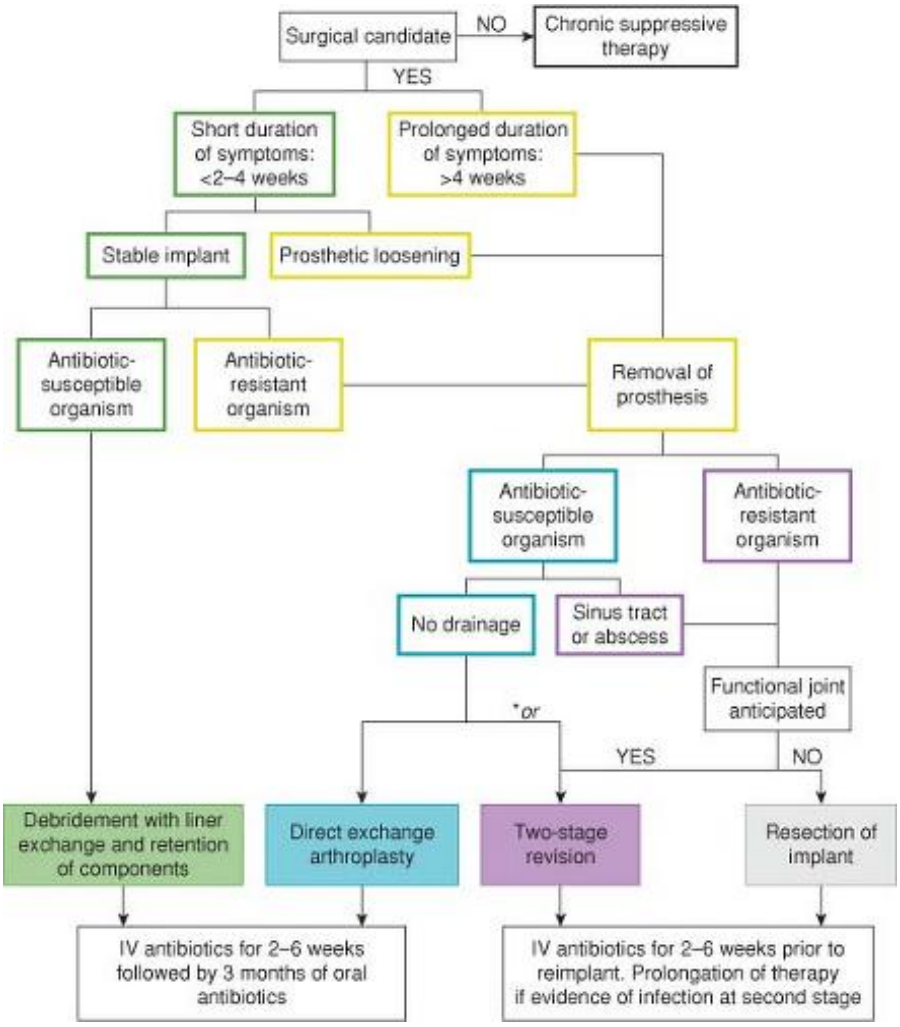

Figure 9. Algorithm for management of prosthetic joint infection of the hip [96]

Fluoroquinolones, doxycycline and sulfamethoxazole/trimethoprim may be treatment options, however, optimal choice depends on several factors including microbiology results. Good oral bioavailability, low risk of side effects and tolerability are essential characteristics of agents considered for long-term treatment. There is a risk of side effects, eg. hypersensitivity (11%), diarrhea (not related to *C. difficile* infection and rather caused by dysbacteriosis) (3-8%) or severe *C. difficile* infection (CDI) including pseudomembraneous colitis (PMC) [98].

The aim of treatment is to eradicate pathogens, provide bactericidal as well as anti-biofilm activity and reduce the risk of resistance development [99]. A study found that

as high as 58.4% of the patients had PJI caused by DTT species with less favourable prognosis and the need of prolonged treatment [52]. This may become difficult in the near future due to the emergence of multiresistant microorganisms. The number of polymicrobial and fungal infections also shows an increasing trend [100]. Another challenge is to achieve therapeutic concentration of antimicrobial agents within the infected tissues and on the surface of implants. Hence biofilm-penetrating antibiotics and antifungal agents play an increasingly important role in the treatment of PJIs.

1.7.2.1. Rifampicin

Rifampicin was discovered in 1965 by the expert group led by Professor Piero Sensi [101]. It inhibits bacterial RNA-polymerase β-subunit, thus mRNA and protein synthesis. Rifampicin is bactericidal and has very long postantibiotic effect. The antibacterial spectrum is broad, mostly covering Gram-positive species as well as Mycobacteria. Bioavailability is excellent as is tissue distribution due to the lipophilic character (Figure 10.). It has exquisite bone penetration and found in bile in high concentration. Rifampicin is metabolised in the liver: being potent enzyme inducer, it can induce the metabolism of several compounds resulting in clinically significant drug interactions. Its efficacy in systemic staphylococcal infections was confirmed in the 1970s [102] and a decade later its potential role in the treatment of implant-assoicated infections was also recognised [103].

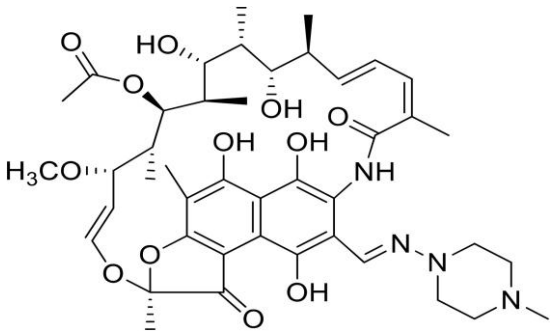

Figure 10. The chemical structure of rifampicin [104]

Antibiotic combinations used in the treatment of PJIs should inhibit the formation of biofilms. The most important antibiotic with anti-biofilm activity is rifampicin, typically used in combination in Gram-positive infections. Similar effects can be attributed to ciprofloxacin exhibiting excellent Gram-negative activity and to penicillin, providing cover for most of Streptococcus spp. However, long-term antibiotic exposure may result in development of antimicrobial resistance. Rifampicin has excellent penetration to biofilms and can achieve bactericidal effect. The use of rifampicin is contra-indicated in monotherapy as resistance can rapidly develop due to a single-step point-mutation. Therefore rifampicin is always used in combination, eg. with fluoroquinolones, doxycycline, sulfamethoxazole/trimethoprim, daptomycin or fusidic acid. Whilst certain centres support the use of the combination of linezolid and rifampicin, some point out the concern of possible interactions and the accelerated metabolism of linezolid potentially resulting in therapeutic failure. Rifampicin as a single agent can only be advised in some rare cases of prophylaxis [105].

Rifampicin can be used in combination against the following pathogens: Staphylococcus spp. [106], Streptococcus spp., possibly Enterococcus spp. [107] and Cutibacterium spp. (having high potential to form biofilm) [108, 109]. The standard dose is 300-600 mg twice a day. According to IDSA guidelines, 2-6 weeks of intravenous treatment (including rifampicin) should be used in the treatment of infections caused by *S. aureus*. The oral stepdown should be considered after 6 weeks of intravenous treatment up to 3 months (6 months if knee joint is involved), however, in some cases 6 months of treatment may be recommended after DAIR procedure [27, 34].

Biofilm diffusion rate of rifampicin depends on the microorganism as well as the age of biofilm. Streptococcus spp. are mostly sensitive to rifampicin and it may show effectiveness against enterococcal biofilms in combination with other antibiotics, however, this effect might be limited to early prosthetic joint infections. Rifampicin also plays a key role in the treatment of mycobacterial infections and may have effect against selected Gram-negative bacteria, particularly biofilm-producing strains [110-115].

It is recommended to initiate rifampicin treatment a few days after the surgery and/or the start of antibiotic treatment. Delay ranges between 3 and 8 days [108]. The rationale behind is that rifampicin has excellent penetration to tissues and various other sites (eg.

abscess, bone) that are less accessible for many other antibiotics. As a consequence, rifampicin would rapidly reach high concentration at the site of infection, however, other agent(s) of the combination may well need longer time to get to steady state. Patient would therefore be on functional rifampicin monotherapy which carries a high risk of rapid development of rifampicin-resistance. The above therapeutic approach aims to avoid this severe consequence [98].

Side effects include nausea, vomiting, abdominal pain and cramps, allergic reaction and orange discolouraction of the urine. This latter is a harmless phenomenon and does not indicate that rifampicin treatment should be ceased. Allergy is rare, however, intolerance may develop especially in high-dose regimes. Hepatotoxicity can lead to deranged liver function tests (LFTs). In such cases dose reduction may be necessary (eg. to 300 mg twice daily) or the use of antiemetics is recommended or 600 mg can be taken once daily in the evening. If these options do not help, 1-2 days of drug holiday may be acceptable and alternative options should be sought.

1.7.2.2. Local antibiotic treatment

Local antibiotic therapy can be considered to achieve higher level of antibacterial effect. This type of administration can result in sustained local concentrations of antibiotics 2-4 times higher than MIC value, facilitating bone and soft tissue penetration. The use of bone cement containing gentamicin resulted in breakthrough in the reduction of the incidence of postoperative complications [9]. Further antibiotics are also available for local treatment, eg. vancomycin and clindamycin. Vancomycin powder can be directly placed in the surgical wound in 1 g or 2 g dose. This glycopeptide antibiotic inhibits the cell wall synthesis of Gram-positive bacteria and exhibitis bactericidal effect. Vancomycin powder proved effective to reduce the risk of infection after hip and knee arthroplasty, however, further studies are required to assess optimal doses [116].

Antibiotic-loaded bone cement (ALBC) was investigated in 2018 from a clinical and cost-benefit perspective. There are several possible ways of administration depending on the chemical composition of the cement, the applied antibiotic and whether it is pre-made or fresh-made during surgery. Clinical approach is not standardised and whilst it is recommended by the British Orthopaedic Association to use ALBC for all knee

arthroplasties, only about 50% of orthopaedic surgeons use cement for primary knee arthroplasty in Canada. Moreover, one-third of them use ALBC for high-risk patients only. A study conducted on 10000 patients undergoing hip arthoplasty has found that the use of ALBC reduced the incidence of PJIs from 0.5% to 0.1%. The cement contained gentamicin in more than 90% of the cases. This study suggests the use of cement for primary and revision total hip arthroplasty (THA), whenever clinically relevant, however, results concerning total knee arthroplasty (TKA) are less evident [117]. Moreover, a study in 2020 pointed out that the application of antibiotic-containing cement is not associated to higher risk of antibiotic resistance [118]. Another approach in 2015 suggested that PJIs can also be prevented by providing antimicrobial coat on the surface of prosthetic material [119].

1.7.2.3. Spacers

Spacers are mostly used in two-stage revisions. After the removal of infected prosthesis, the new device can not be implanted until the inflammation of the surrounding tissues settles. Spacers are used to bridge anatomical distances, reduce the risk of intra- and periarticular haematoma and maintain local mechanical stability. Dinamic spacers also allow functional movements whereas they are restricted by static spacers [120]. However, in a joint with ongoing polymicrobial infection, multiple previous surgical procedures and developing osteomyelitis, static spacers may provide better healing rates. Stimulan uses calcium-sulphate crystals to be a vehicule of vancomycin powder for local treatment. Hand-made antibiotic-contaning balls can be formed intraoperatively and then placed into the infected joint providing effective local antibiotic concentration at the surgical site. Side effects include hypercalcaemia, ongoing wound discharge and heterotropic ossification, however, they were found to be rare [121].

DOI:10.14753/SE.2024.2998

2. Objectives

It is fundamentally important to recognise the signs and symptoms of a developing prosthetic joint infection in order to start conservative and/or surgical management as early as possible. On the other hand, it also crucial to assess the effect of various factors on the outcome of prosthetic joint infections. For this reason risk factors need to be identified. When relevant risk factors are recognised and determined, it is possible to perform risk assessment for the recovery rates of patients. Not only this approach is extremely useful for patients after the implantation of prosthetic device in the follow up period but it can also help finding the most appropriate route of implantation before the procedure.

We investigated the effects of various factors on the recovery rates of patients with prosthetic joint infection in two groups with significantly different medical approaches: patients undergoing two-stage revision and patients undergoing DAIR procedure. In the first group it is not possible to save the prosthesis, hence all components of the implant must be removed and replaced with a new device. In the second group, however, the fix parts of the prosthesis remain *in situ* after appropriate debridement.

I. Our first aim was to investigate the effect of risk factors in patients undergoing two-stage revision and identify those having significant clinical impact on recovery rates.

Specific questions:

1., Which patient-related and -unrelated factors have significant impact on recovery rates? How can the impact be characterised?

2., How does rifampicin-resistance of the causative agent influence recovery rates?

II. The second aim was to investigate the effect of risk factors in patients undergoing DAIR procedure and identify those having significant clinical impact on recovery rates.

Specific questions:

1., Which patient-related and -unrelated factors have significant impact on recovery rates? How can the impact be characterised?

2., How does rifampicin-resistance of the causative agent influence recovery rates?

3., Is there any correlation between rifampicin-resistance and orthopaedic risk stratification scores such as KLIC and CRIME80?

III. The clinical significance of the isolated microorganism(s) must always be carefully assessed. The optimal antimicrobial therapy can only be established by considering microbiological results, the characteristics of the infection and patient's conditions. The following investigations have been carried out among patients with two-stage revision as well as those undergoing DAIR procedure.

Specific questions:

1., What is the prevalence of different microorganisms in the rifampicin-sensitive and in the rifampicin-resistant group? Is there any characteristic difference between two-stage revision and DAIR patients?

2., Are there polymicrobial infections recognised?

3., Is there any evidence for the development of rifampicin-resistance during antimicrobial therapy?

4., Is rifampicin-resistance related to previous rifampicin-based regimes?

5., What antimicrobial regimes are used in the treatment of patients with prosthetic joint infection? Were they appropriate in every aspect?

IV. After collecting and reviewing data we aimed to draw conclusions in relation to clinical practice.

Specific questions:

1., Which factors should be considered when estimating recovery rates?

2., Which factors are associated with higher recovery rates? Can these factors be influenced?

3., How can various factors help the decision as to what type of orthopaedic method should be preferred?

DOI:10.14753/SE.2024.2998

3. Methods

3.1. Study population

Patients were followed up 6 weeks, 3 months and 6 months after surgery and on a yearly basis thereafter. However, if clinically indicated, patients were re-assessed more frequently. Recovery was considered in patients with no clinical, radiological and laboratorical signs of infection after a follow up period of two years. After statistical description of our data, we reviewed recovery rates among patients and investigated the effect of rifampicin-resistance and patient-related factors.

3.1.1. Patients undergoing two-stage revision

Our study was approved by the Ethics Committe of Semmelweis University and carried out in accordance with the Declaration of Helsinki. All patients gave their informed consent and were anonymised. We retrospectively reviewed the medical records of 73 patients (41 males and 32 females) admitted to the Department of Orthopaedics, Semmelweis University undergoing two-stage revision due to low-grade PJI between 2017 and 2019.

Past medical history, risk factors, comorbidities and clinical details were collected and analysed. Short-term and long-term outcome, previous surgical and antibiotic therapies were also reviewed. Sex, ASA score and clinical conditions such as hypertension, chronic heart failure, chronic renal failure (CRF), chronic pulmonary diseases, type 1 and 2 diabetes mellitus (DM), haematological and thyroid disorders, liver cirrhosis, stroke and rheumatoid arthritis (RA) were investigated. Patients were classified by body mass index according to the WHO score system. Participants were divided into two groups according to rifampicin sensitivity result(s) of the microorganism(s) causing PJI.

3.1.2. Patients undergoing DAIR procedure

Our study was approved by the Ethics Committe of Semmelweis University and carried out in accordance with the Declaration of Helsinki. All patients gave their informed

consent and were anonymised. We retrospectively reviewed the medical records of 67 patients (37 males and 30 females) admitted to the Department of Orthopaedics, Semmelweis University undergoing DAIR procedure due to early onset PJI (starting within 6 weeks after the index surgery according to the International Consensus Meeting on Musculoskeletal Infections meeting criteria [65]) between 2014 and 2021.
Past medical history, risk factors, comorbidities and clinical details were collected and analysed. Factors included the affected joint, previous trauma, treatment duration, antibiotic regime(s), administration of jet lavage, exchange of mobile elements and revision before and after DAIR procedure. Sex, age, comorbidities including diabetes mellitus, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, chronic renal failure, liver cirrhosis, thyroid diseases, hypertension and coagulation abnormalities as well as ASA score and BMI were reviewed. In our cross-sectional study patients were divided into two groups according to rifampicin sensitivity result(s) of the microorganism(s) causing PJI.

3.2. Score systems

Based on our data, CRIME80 and KLIC scores were calculated preoperatively to estimate the risk of failure of DAIR procedure and the recurrence of PJI. Obtained scores can be used to select patients not suitable for DAIR procedure and cases when the efficacy and outcome of treatment are doubtful. Of note, these score systems are not appropriate for patients undergoing two-stage revision. The characteristics of KLIC and CRIME80 score systems are detailed in Figure 11. and 12.

3.3. Microbiological background

Clinical specimens were processed in the Clinical Microbiological Diagnostic Laboratory (Institute of Laboratory Medicine, Semmelweis University) with conventional methods including microscopy, culture and antibiotic sensitivity testing (disk diffusion and E-tests according to the European Committee on Antimicrobial Susceptibility Testing [EUCAST] guidelines). Microbiology reports (including antibiograms) were collected and the clinical significance of each isolate was assessed.

Acronym	Definition	Points
K	Chronic kidney failure	2
L	Liver cirrhosis	1.5
I	Arthroplasty indication: fracture OR revision	1.5
C	Cemented implants CRP > 115 mg/L	2 2.5

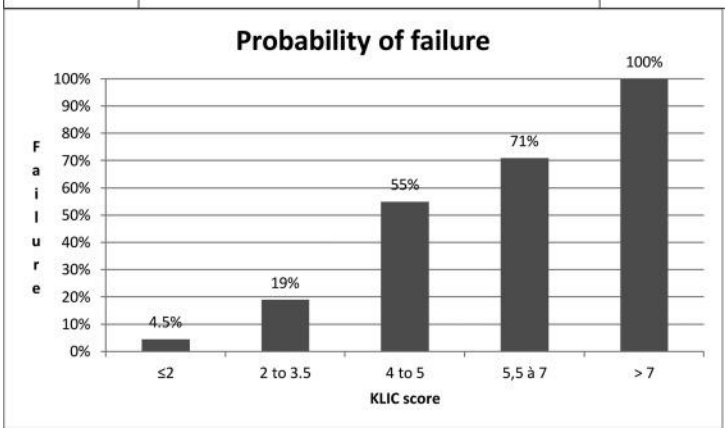

Figure 11. The KLIC score [122]

Acronym	Definition	Points
C	Chronic obstructive pulmonary disease CRP > 150 mg/L	2 1
R	Rheumatoid arthritis	3
I	Arthroplasty indication: fracture	3
M	Male	1
E	Exchange of mobile components	-1
80	Age > 80 years	2

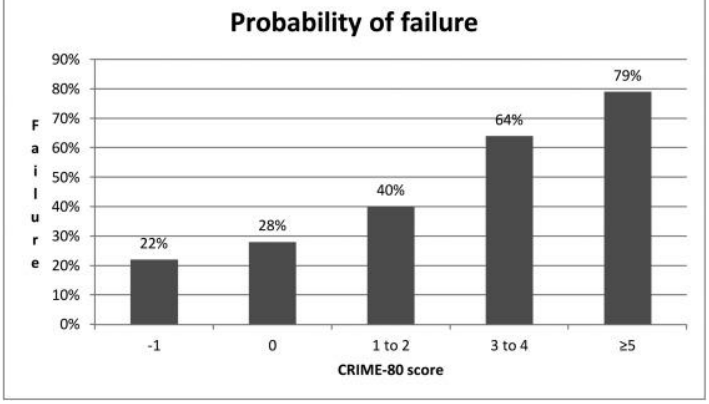

Figure 12. The CRIME80 score [122]

The most frequent types of specimens were punctures and aspirates (either cultured directly or incubated in blood culture bottles) as well as intraoperative deep biopsy samples (eg. periprosthetic tissues) and swabs. Direct culture is the gold standard method in most cases, however, causative agent(s) may be missed if they are only present in low germ count (eg. <10 CFU/mL) in the original specimen, ie. at the site of

infection. Enrichment of the microorganisms is required in such cases by the use of various enrichment solutions. Blood cultures also belong to this group of media with the aim of increasing the concentration of the pathogen causing bloodstream infection by facilitating its proliferation. However, it can also be used for the enrichment of other liquid specimens with the same consideration.

Significance can be assessed by the characteristics of the microorganism ("does it have the potential to cause PJI?"), the clinical picture ("can this isolate be related to the clinical symptoms?"), the number of specimens it was isolated from ("was it isolated from 1 or 5 samples?"), the types of specimens it was isolated from ("was it isolated from superficial swabs or deep tissue samples") and whether it was isolated from direct culture or from enrichment only (referring to the germ count of the isolate in the original specimen and at the site of infection). Whenever mixed cultures are obtained (ie. at least two microorganisms are present *in vitro*), the determination of significance can sometimes be even more challenging. Whilst the detection of further microorganim(s) by itself does not change the role of the isolate in question and polymicrobial (mixed) infections can also occur, a heavily mixed culture may indicate contamination of the original specimen, eg. with normal skin flora and/or due to inappropriate sampling techniques. This can result in suboptimal treatment and therapeutic failure or recurrence of the infection. We have investigated polymicrobial infections in patients undergoing two-stage revision as well as those undergoing DAIR procedure. In such cases, patients were placed in the resistant group if at least one of the significant isolates was rifampicin-resistant.

The antibiotic susceptibility pattern was reviewed for all significant pathogens including multidrug resistant organisms (MDROs). Isolates were divided into two groups based on their rifampicin sensitivity. Whenever not tested, *Cutibacterium* (formerly *Propionibacterium*) *acnes* and Streptococcus spp. were cathegorised as rifampicin-sensitive whereas Enterococcus spp., *Enterobacterales* and *Pseudomonas aeruginosa* as rifampicin-resistant according to their inherited resistance profile and expert rules. Development of rifampicin-resistence during treatment was investigated by comparing the antibiotic sensitivity profile of the same microorganism in different specimens taken during the course of infection.

We also reviewed antimicrobial regimes used to treat PJIs including choice of antimicrobial agent(s) as well as route, dose and duration of therapy. These data are crucially important to assess the appropriateness of treatment. It was also investigated whether patients had received rifampicin prior to their current orthopaedic infection. Previous exposure to rifampicin increases the risk of resistance development during treatment as well as the presence of microorganism(s) already resistant to rifampicin.

3.4. Statistical analysis

The statistical analysis was performed by using the R software (R Core Team 2022) [123] and its ggplot2 package for figures [124]. After describing data, a logistic regression model was fitted: we used recovery rate as the outcome and rifampicin-resistance as the explanatory variable. The effect was controlled for sex, age, BMI and DM as possible or known confounders. After taking into consideration possible multicollinearity (based on graphs and variance inflation factor [vif] values) and possible interactions (based on common sense, graphs, model fit diagnostics and information criteria), the interaction effect between rifampicin-resistance and age was also included in the final model. The model fit was acceptable based on model diagnostic plots. Decisions were made on null-hypothesis using 5% as significance level. No multiplicity correction was made.

Since our study has an observational study design, it was crucially important to adjust the effect of rifampicin-resistance for potential confounders and assess the effect modification of interested variables. Therefore we used a regression model. The aim of our model was to investigate the effect of most interested variables on the given clinical outcome in order to implement a sample interpretable model (not a prediction model). Due to the limited sample size, we considered how many and which predictors to include in our final model. As a "rule of thumb" the number of predictors (more precisely the fitted predictor parameters, i.e. slopes) should be at about maximum 1/10 times the sample size [125].

DOI:10.14753/SE.2024.2998

4. Results

4.1. Patients undergoing two-stage revision

4.1.1. Rifampicin-resistance and patient-related factors

The overall recovery rate was 83.6% (61 out of 73 patients), 96.5% among patients within the rifampicin-sensitive group and 60.0% in the resistant group, as we have previously published [126]. The mean age was 68.8 years (standard deviation (SD) = 10.8 years) and the mean body mass index (BMI) was 30.2 kg/m^2 (SD = 5.13 kg/m^2). 15 patients (20.5%) had type 2 diabetes mellitus (DM) and none had type 1. 48 patients had hip, 22 had knee, 2 had shoulder and 1 had elbow joint infection. Selected data of the study population are summarised in Table 1.

According to our statistical analysis the following variables had significant impact on recovery rates: rifampicin-resistance, age, sex and type 2 diabetes mellitus, however, we found no clear evidence for the effect of BMI (Table 2.). We also reviewed further possible risk factors affecting recovery including chronic heart diseases, hypertension, ASA score, stroke, chronic lung diseases, chronic renal failure, thyroid diseases and haematology disorders, however, we could not draw statistically relevant conclusions due to the low number of patients with certain risk factors. On the other hand, recovery rates were found significantly higher in male patients.

22.6% of our patients had type 2 diabetes mellitus in the sensitive and 15.0% in the resistant group. We analysed the correlation between recovery rates and age, then compared this correlation between nondiabetic and diabetic patients and reviewed the differences. Age had a remarkable impact on recovery rates in the rifampicin-sensitive group but this effect was found minimal in the resistant group (Figure 13.). This can be observed in both nondiabetic and diabetic patients, however, the recovery rates in the presence of rifampicin-resistant microorganism were significantly lower in diabetic patients. In the rifampicin-sensitive group the recovery rates are close to 100% in younger individuals regardless of diabetes mellitus. The kinetics is also similar: after an age-threshold there is a significant declination in recovery approaching 0% in higher age groups. Another important difference between nondiabetic and diabetic patients has

to be highlighted: the declination in recovery rates starts at the age of 70 years and reaches 50% after 80 years in the nondiabetic group, whereas the same events occur approximately 10 years earlier in the diabetic group (start of declination at 60 years, 50% recovery at 70 years of age).

Table 1. Summary of study population data used for statistical analysis (patients undergoing two-stage revision) (table from the candidate's publication [126])

	Rifampicin Sensitive (N=53)	**Rifampicin Resistant (N=20)**	**Overall (N=73)**
Recovery			
Recovered	49 (92.5%)	12 (60.0%)	61 (83.6%)
Not-recovered	4 (7.5%)	8 (40.0%)	12 (16.4%)
Sex			
Male	31 (58.5%)	10 (50.0%)	41 (56.2%)
Female	22 (41.5%)	10 (50.0%)	32 (43.8%)
Age [years]			
Mean (SD)	68.6 (10.1)	69.4 (12.7)	68.8 (10.8)
Median (IQR)	70.4 (8.49)	71.1 (16.6)	70.4 (10.9)
Min, Max	35.0, 86.3	39.3, 87.9	35.0, 87.9
Diabetes mellitus			
Non-diabetic	41 (77.4%)	17 (85.0%)	58 (79.5%)
Diabetic	12 (22.6%)	3 (15.0%)	15 (20.5%)
BMI [kg/m^2]			
Mean (SD)	29.8 (4.88)	31.4 (5.73)	30.2 (5.13)
Median (IQR)	29.0 (6.00)	32.5 (9.63)	29.7 (6.80)
Min, Max	21.1, 44.9	20.3, 38.0	20.3, 44.9

In a different comparison, the impact of rifampicin-resistance was more pronounced among younger patients: recovery rates were higher in the rifampicin-sensitive group at lower ages, however, the difference was decreasing with advancing age. The effect of rifampicin-resistance on recovery rates was more dramatic in diabetic patients. On the

other hand, as recovery rates decline with age in the rifampicin-sensitive group (regardless of diabetes), the effect of rifampicin-resistance decreases and even disappears at higher ages. Overall, the poorest outcomes can be expected in elderly diabetic patients with rifampicin-resistant isolate. These findings suggest significantly negative effect of age, diabetes mellitus and rifampicin-resistance on clinical outcomes.

Table 2. Effect estimates of risk factors with its 95% confidence interval based on a regression model (patients undergoing two-stage revision) (table from the candidate's publication [126])

Predictors	**Odds ratio (recovered vs. not recovered)**	**95% confidence interval**	**p-value**
(intercept)	29.4181	11.2922 – 54.7758	0.0063
Rifampicin-resistance: Resistant	(-)25.1947	(-)49.2322 – (-)8.5634	0.0109
Age 1 year	(-)0.2985	(-)0.5918 – (-)0.0921	0.0143
Sex: Male	2.3837	0.5985 – 4.6502	0.0176
BMI 1 kg/m^2	(-)0.1557	(-)0.3669 – 0.0097	0.0906
Diabetes mellitus: Diabetic	(-)2.9763	(-)5.9344 – (-)0.6499	0.0217
Rifampicin-resistance and Age interaction: Resistant: 1 year	0.3052	0.0845 – 0.6159	0.0181

Most of the patients had previous surgery of the affected joint: 49 out of 53 (92.5%) in the sensitive group and all patients in the resistant group. 9.4% of the patients (5 out of 53) had fracture as a risk factor for PJI in the rifampicin-sensitive and 30.0% (6 out of 20 patients) in the -resistant group.

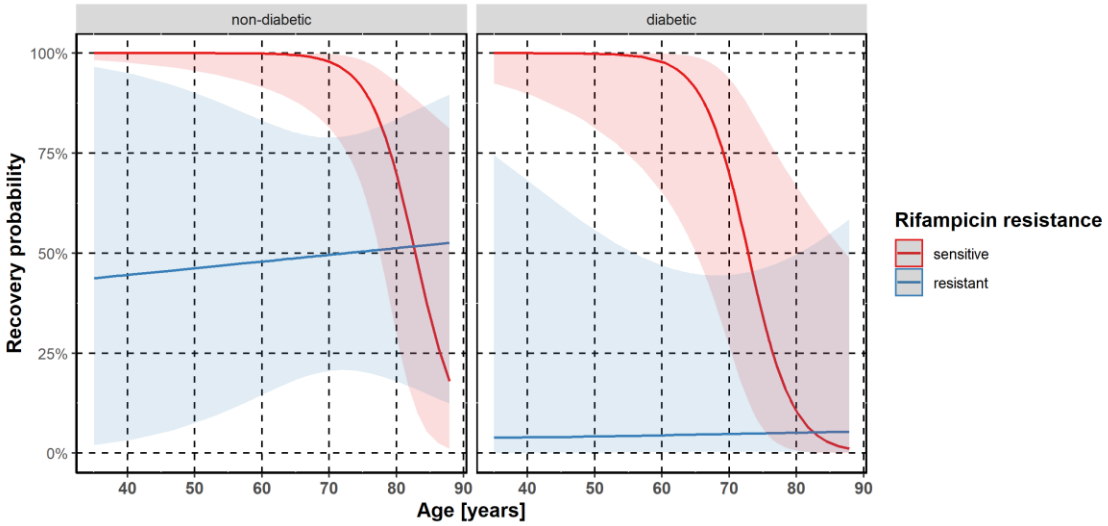

Figure 13. Predicted recovery probability based on a regression model (for female, at mean BMI) (patients undergoing two-stage revision) (figure from the candidate's publication [126])

4.1.2. Microbiological background

We reviewed the microbiology reports of all patients included in this study [126]. Whenever not tested, *Cutibacterium* (formerly *Propionibacterium*) *acnes* and Streptococcus spp. were cathegorised as rifampicin-sensitive whereas Enterococcus spp., *Enterobacterales* and *Pseudomonas aeruginosa* as rifampicin-resistant. Fifty-three out of 73 patients (72.6%) had PJI caused by rifampicin-sensitive and 20 (27.4%) by rifampicin-resistant microorganism. We found that rifampicin-sensitive species was isolated in 80.3% among recovered patients and in 33.3% in patients with treatment failure (Figure 14.).

According to our results, Staphylococcus spp. were predominant in the sensitive group (66.7% of the isolates), most of which were coagulase-negative Staphylococcus spp. (27 isolates). *S. aureus* was isolated in 9 cases including one MRSA strain. Among CNS, the following species were cultured: *S. epidermidis* (13 cases) followed by *S. hominis* (7 cases), *S. haemolyticus* (2 cases), *S. capitis* (1 case) and not further identified CNS species (2 cases). Of note, *S. lugdunensis* was also isolated in two cases: its importance is highlighted by the potential of this species to cause infections as severe as those caused by *S. aureus*. The majority of Staphylococcus spp. were sensitive to rifampicin:

9 out of 10 *S. aureus* and 27 out of 32 coagulase-negative Staphylococcus spp. *Cutibacterium acnes* was cultured in 8.2% of the patients (6 cases). Although *C. acnes* has been recognised as a possible causative agent of PJIs with the ability of biofilm formation, in certain cases it may be difficult to assess clinical significance as it is part of normal skin flora [127]. An unusual pathogen, *Arthrobacter scleromae* was isolated in one case. *Streptococcus agalactiae* was the predominant streptococcal isolate in our patients (7 out of 11 cases), followed by *S. gallolyticus* (2 cases) and *S. anginosus* (1 case). In one case *S. pneumoniae* was isolated. *Haemophilus parainfluenzae* was the causative agent in one case, highlighting the clinical importance of this species.

The pathogen distribution was significantly different in the rifampicin-resistant group. Gram-negative rods (including *Enterobacterales* and nonfermenters) and Enterococcus spp. represented the majority of isolates. Most of the Gram-negative rods belonged to *Enterobacterales* order ("coliforms"): *Escherichia coli* was isolated in 2 cases followed by *Klebsiella oxytoca* (1 case), *Enterobacter cloacae* (1 case), *Serratia marcescens* (1 case), *Proteus mirabilis* (2 cases) and *Morganella morganii* (1 case). One *Pseudomonas aeruginosa* isolate represented nonfermenters. *E. faecalis* was isolated in 5 cases (9.6% of the patients), *E. casseliflavus* and *E. faecium* in 1 case each. The latter turned out to be an acquired vancomycin-resistant Enterococcus (VRE) strain. Of note, Enterococcus spp. show increasing prevalence in the etiology of PJIs [57, 128]. One *S. aureus* (MSSA) and 5 coagulase-negative Staphylococcus spp. (all identified as *S. epidermidis*) were isolated. A rifampicin-resistant strain of *Corynebacterium striatum* and *Mycobacterium goodii/smegmatis* was also found. Four patients had polymicrobial infection in the sensitive and one in the resistant group. On the other hand, we recognised no remarkable changes in the pathogen distribution during the 3-year period of our study.

Of note, 15.0% of the patients had previous rifampicin treatment in the resistant group (all of them in combination with other antibiotics, in most of the cases with vancomycin or sulfamethoxasole/trimethoprim) and none in the sensitive group. Rifampicin has the potential to loose its clinical efficacy due to rapid antibiotic resistance development. We observed the development of rifampicin-resistance in three cases of PJI: once caused by *S. aureus* (MSSA) and twice by *S. epidermidis* (both MRSE). The first patient was initially treated with intravenous cefazolin followed by oral linezolid + rifampicin and

later on switched to levofloxacin + rifampicin. Both patients with MRSE infection were treated with intravenous vancomycin and oral rifampicin. After the IV session, patient was switched to PO doxycycline in one case and to PO linezolid in the other case, both of them given in monotherapy. The risk of resistance development towards rifampicin may also increase if the combination partner has limited activity on Gram-positive bacteria (eg. ciprofloxacin).

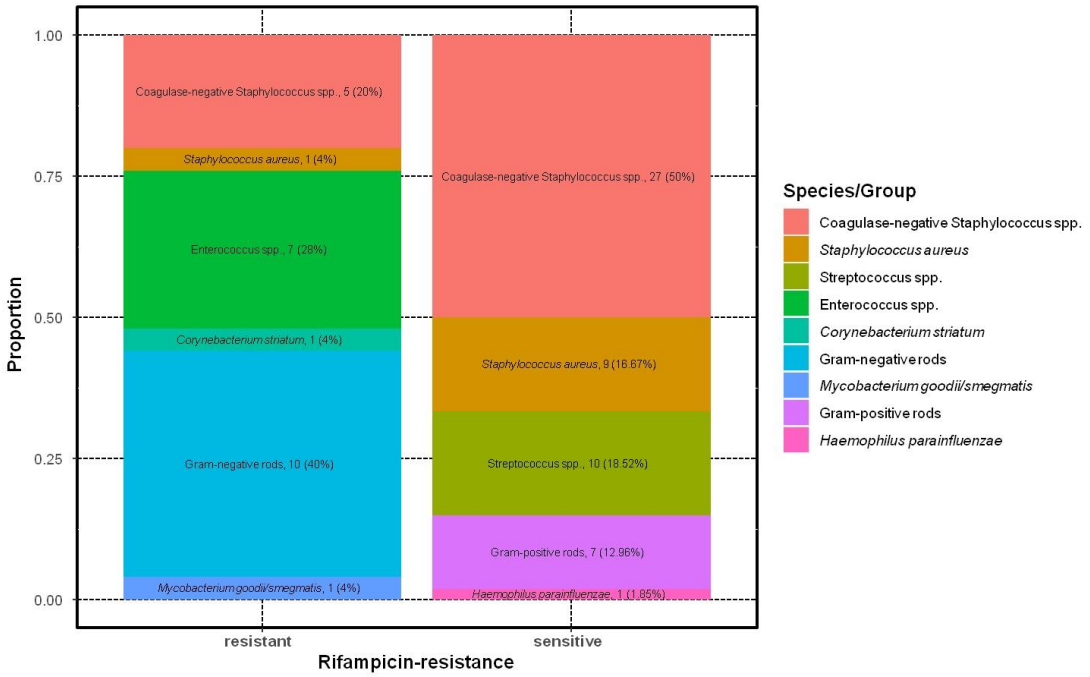

Figure 14. Pathogen distribution of rifampicin-resistant and -sensitive isolates (count, percentage) (patients undergoing two-stage revision)
(figure from the candidate's publication [126])

4.2. Patients undergoing DAIR procedure

4.2.1. Rifampicin-resistance and patient-related factors

47 (70.1%) patients had rifampicin-sensitive and 13 (19.4%) patients had -resistant isolate, as we have previously published [129]. The overall recovery rate was 74.6% (50 out of 67 patients). Interestingly, recovery rate was 72.3% among patients within the rifampicin-sensitive and 76.9% in the resistant group. Altogether 15 patients (22.4%) had therapeutic failure. Significant pathogens were isolated in 60 out of 67 cases. The mean age was 68.4 years (standard deviation (SD) = 15.8 years) and the mean body

mass index was 30.5 kg/m^2 (SD = 5.87 kg/m^2). Among recovered patients mean age was lower in the sensitive group (65.6 vs 73.7 years), however, the opposite was observed among patients without recovery (71.9 vs 57.8 years). 11 patients (16.4%) had diabetes mellitus (DM): 2 had type 1 and 9 had type 2. 44 patients had hip, 21 had knee, 1 had shoulder and 1 had elbow joint infection. Selected data of the study population are summarised in Table 3.

Table 3. Summary of study population data used for statistical analysis (patients undergoing DAIR procedure) (table from the candidate's publication [129])

	Not recovered (N=17)	**Recovered (N=50)**	**Overall (N=67)**
Rifampicin-resistance			
Sensitive	13 (76.5%)	34 (68.0%)	47 (70.1%)
Resistant	3 (17.6%)	10 (20.0%)	13 (19.4%)
No data available	1 (5.9%)	6 (12.0%)	7 (10.4%)
Sex			
Male	10 (58.8%)	27 (54.0%)	37 (55.2%)
Female	7 (41.2%)	23 (46.0%)	30 (44.8%)
Age [years]			
Mean (SD)	69.4 (15.8)	68.1 (18.0)	68.4 (15.8)
Median (IQR)	73.7 (13.2)	72.6 (14.8)	72.8 (14.4)
Min, Max	26.9, 86.7	28.5, 86.7	26.9, 86.7
Diabetes mellitus			
Non-diabetic	13 (76.5%)	43 (86.0%)	56 (83.6%)
Diabetic	4 (23.5%)	7 (14.0%)	11 (16.4%)
BMI [kg/m^2]			
Mean (SD)	30.1 (5.21)	30.7 (6.12)	30.5 (5.87)
Median (IQR)	27.6 (4.99)	29.4 (8.20)	29.0 (7.85)
Min, Max	24.3, 42.4	18.7, 46.9	18.7, 46.9
No data available	0 (0%)	1 (2.0%)	1 (1.5%)

We investigated the effect of selected factors such as rifampicin-resistance, sex, age, DM and BMI in a multivariate regression model. Although we observed some correlations, according to our analysis we found no statistically significant effect of these variables on recovery rates (Table 4.). We also reviewed further risk factors including hypertension, ASA score, C-reactive protein (CRP), COPD, liver cirrhosis, CRF, haematology disorders, RA and whether implant included cement, however, we could not perform statistical inferential analysis due to the low number of patients with certain risk factors.

Table 4. Effect estimates of risk factors with its 95% confidence interval based on a regression model (patients undergoing DAIR procedure) (table from the candidate's publication [129])

Predictors	**Odds ratio (recovered vs. not recovered)**	**95% confidence interval**	**p-value**
(intercept)	7.5069	0.1021 – 803.8825	0.3697
Rifampicin-resistance: Resistant	1.2372	0.3046 – 6.3043	0.7766
Age 1 year	0.9892	0.9478 – 1.0259	0.5801
Sex: Male	0.7513	0.2048 – 2.5711	0.6534
BMI 1 kg/m^2	1.0003	0.8985 – 1.1203	0.9956
Diabetes mellitus: Diabetic	0.3948	0.0876 – 1.8473	0.2206

We can assume a clinically important impact of age on recovery rates both in the rifampicin-sensitive and in the resistant group (Figure 15.), although it may prove difficult to assess the significance of this effect due to the relatively low number of patients and possible unknown confounders. We also observed the negative clinical effect of diabetes mellitus on recovery rates in our cohort. There was no significant difference in recovery rates between the rifampicin-sensitive and -resistant group

regardless of diabetes mellitus. Instead of a rapid declination above an age-threshold as experienced in patients undergoing two-stage revision, there is a continuous slow decrease in recovery rates with advancing age among patients with DAIR procedure. Diabetes has only a limited negative effect on outcomes in both the rifampicin-sensitive and -resistant group. These findings are significantly different from those seen in patients undergoing two-stage revision.

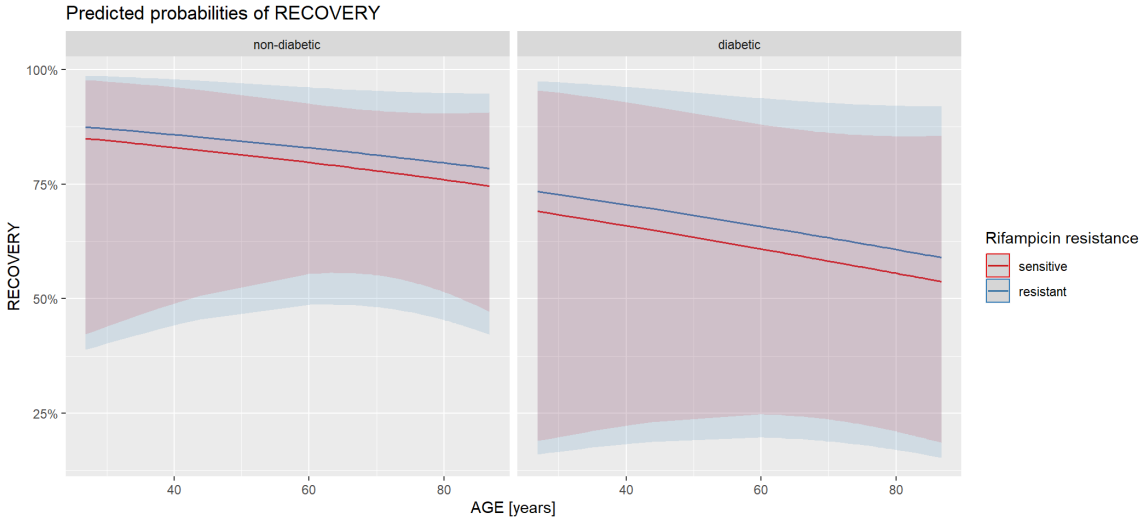

Figure 15. Predicted recovery probability based on a regression model (for female, at mean BMI with 95% confidence interval) (patients undergoing DAIR procedure) (figure from the candidate's publication [129])

It has been demonstrated that injury or surgery of the joint capsule in the hip and knee significantly increases the risk of PJI. Six patients (46.2%) in the rifampicin-sensitive group had surgery of the involved joint before and 26 out 47 patients (55.3%) in the resistant group. We also investigated whether patients had two-stage revision due to PJI before and/or after DAIR procedure. Our findings are summarised in Table 5.

25.5% of the patients in the sensitive group had two-stage revision before DAIR procedure and 38.5% in the resistant group. However, there was no clinically relevant difference in the rates between the two groups after DAIR (19.1% and 15.4%). We also investigated how revision before DAIR influenced recovery rates. There were 12 revisions in the rifampicin-sensitive group with 66.6% recovery rate and 5 revisions in the resistant group with 80.0% recovery. Without previous revision the recovery rates

were 74.3% and 75.0%. As a conclusion, there was no statistically significant effect of previous revision on the outcome in patients undergoing DAIR procedure.

Table 5. The number of two-stage revisions before and after DAIR procedure

	Revision before DAIR procedure	**Revision after DAIR procedure**	**Revision before and after DAIR procedure**
Rifampicin-sensitive	11	8	1
Rifampicin-resistant	4	1	1

The exchange of mobile elements was also reviewed: 16 patients had head exchange, 19 had insert exhange, 2 had both and 30 had none. Altogether 55.3% of the patients had exchange in the sensitive and 46.2% in the resistant group. Insert exchange was less whereas head exchange and no exchange were more predominant in the resistant group (Table 6.). Insert exchange resulted in higher recovery rate and by the exchange of more components an even higher rate was achieved in the rifampicin-sensitive group. When no exchange was performed, recovery rates were identical in the two groups (71.4%).

Table 6. Exchange of mobile elements in patients undergoing DAIR procedure

	Head exchange	**Insert exchange**	**Both**	**None**
Rifampicin-sensitive	9	14	3	21
Rifampicin-resistant	4	2	0	7

We also reviewed the correlations between CRIME80 and KLIC score systems and recovery rates in the rifampicin-sensitive and -resistant group. Recovery rates were compared among patients with CRIME80 score ≥3 (19 cases) (Figure 16.). 100% recovery was seen in the resistant and 66.7% in the sensitive group. Average score of recovered patients in the sensitive group was 1.65, whereas it was 2.1 in the resistant group. Average KLIC score of recovered patients in the sensitive group was 2.91 and

2.85 in the resistant group. Nineteen patients had higher than 4.5 in KLIC score. 2 out of 2 patients recovered in the resistant group and recovery rate was 70.6% in the sensitive group. 92.3% of the patients had KLIC score in the 2-3.5 range in the resistant group. Although the same range was predominant in the sensitive group (42.6%), the distribution was more balanced (Figure 17.). Altogether we found no significant effect of CRIME80 and KLIC score on recovery rates neither in the rifampicin-sensitive nor in the -resistant group.

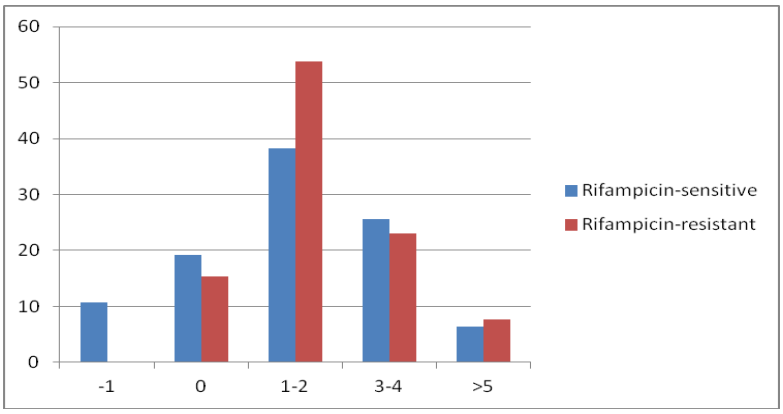

Figure 16. Distribution of CRIME80 score results in the rifampicin-sensitive and -resistant group

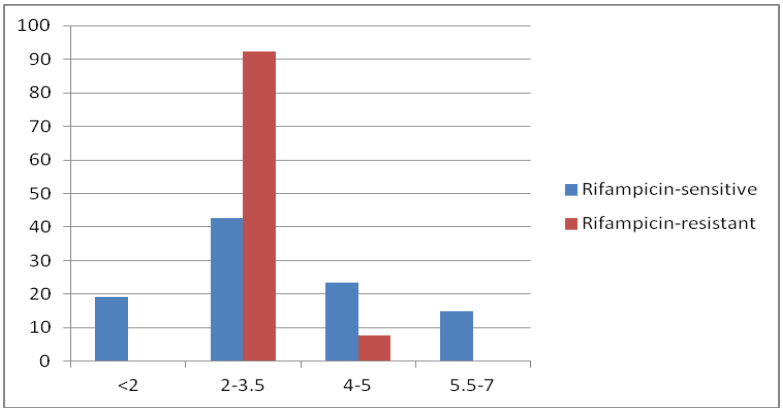

Figure 17. Distribution of KLIC score results in the rifampicin-sensitive and -resistant group

4.2.2. Microbiological background

We reviewed the microbiology reports of all patients included in this study [129]. Similarly to the group of patients undergoing two-stage revision, whenever not tested, *Cutibacterium* (formerly *Propionibacterium) acnes*, *Finegoldia magna* and

Streptococcus spp. were cathegorised as rifampicin-sensitive whereas Enterococcus spp., *Enterobacterales* and *Pseudomonas aeruginosa* as rifampicin-resistant according to their natural resistance profile and expert rules. Fourty-seven out of 67 patients (70.1%) had PJI caused by rifampicin-sensitive and 13 (19.4%) by rifampicin-resistant microorganism. We found that rifampicin-sensitive species was isolated in 77.3% among recovered patients and 81.3% in patients with treatment failure (Figure 18.).

Staphylococcus spp. were predominant in the rifampicin-sensitive group (66.7% of the isolates) including 18 *S. aureus* and 18 coagulase-negative Staphylococcus spp. with the predominance of *S. epidermidis* (10 isolates). Other species included *S. haemolyticus* (3 cases), *S. capitis*, *S. cohnii*, *S. simulans* and *S. warneri* (1 case each). Of note, *S. lugdunensis* was isolated in one case, having the potential to cause severe infections similarly to *S. aureus*. Among Streptococcus spp., *S. dysgalactiae* was isolated in 5 cases, *S. agalactiae* in 4 cases and *S. parasanguinis* in 1 case. Of note, *S. pneumoniae* was isolated in one case. Similarly to patients undergoing two-stage revision, β-haemolytic species were predominant among Streptococcus spp. *C. acnes* was found to be the causative agent in six cases.

The pathogen distribution was significantly different in the rifampicin-resistant group: Staphylococcus spp. were less prevalent (2 *S. aureus* isolates). Instead, Gram-negative rods (12 *Enterobacterales* and 2 *P. aeruginosa* strains) as well as Enterococcus spp. (5 *E. faecalis* isolates) were predominant. Among *Enterobacterales*, *Escherichia coli* was the most frequent species (6 cases). Other pathogens included *Enterobacter cloacae* (2 cases), *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*), *Klebsiella oxytoca*, *Serratia marcescens* and *Providencia rettgeri* (1 case each). A rifampicin-resistant strain of *Arthrobacter polychromogenes* and *Mycobacterium goodii/smegmatis* was also isolated. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection itself is a risk factor resulting in more difficult eradication of PJIs [59]. The incidence of multiresistant organisms were low: 3 out of 20 *S. aureus* isolates were MRSA. Development of rifampicin-resistance was not observed in our cohort and none of the patients had received previous rifampicin treatment. Three patients had polymicrobial infection in the sensitive and four in the resistant group. Gram-positive bacteria were involved in all these cases and 70.0% of the pathogens were resistant to rifampicin.

Antibiotic regimes were also reviewed in our study. Rifampicin administration was documented in 34 cases in combination with other antibiotics depending on microbiology results and patient-related factors. Of note, rifampicin was used in monotherapy in two cases. Combination partners included ciprofloxacin, levofloxacin, sulfamethoxazole/trimethoprim, doxycycline and amoxicillin. In one case, a triple therapy of amoxicillin, ciprofloxacin and rifampicin was established. When comparing antibiotic regimes, ciprofloxacin was the combination partner when the highest number of rifampicin-resistant microorganisms were isolated (30.8%). The most frequent combinations in the sensitive group were sulfamethoxasole/trimethoprim + rifampicin (19.1%) and levofloxacin + rifampicin (17.0%).

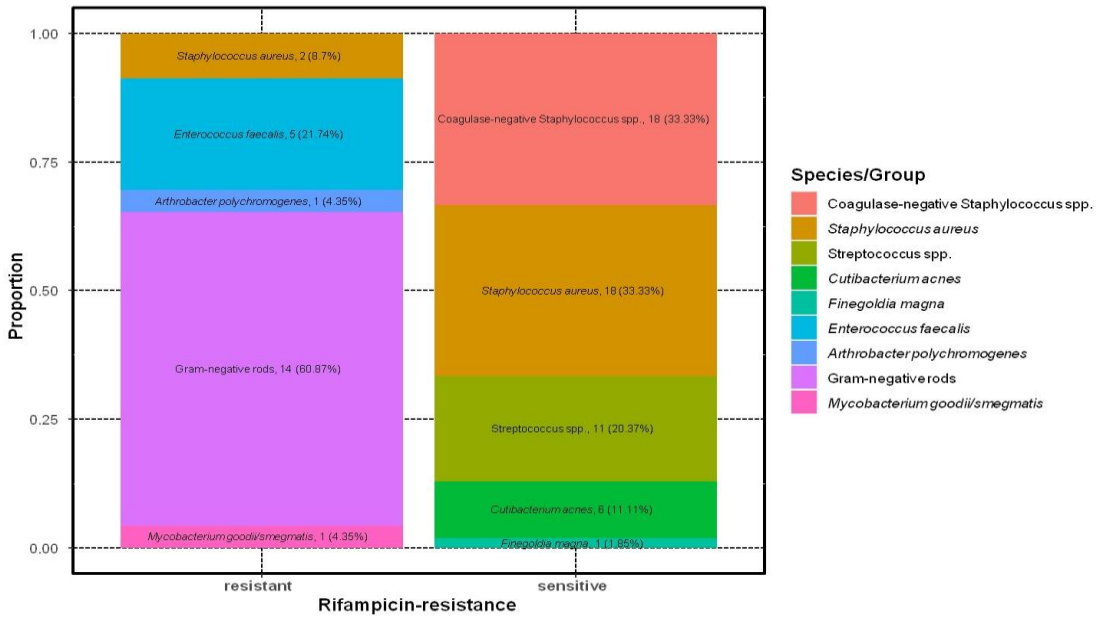

Figure 18. Pathogen distribution of rifampicin-resistant and -sensitive isolates (count, percentage) (patients undergoing DAIR procedure)
(figure from the candidate's publication [129])

It is also interesting to note the recovery rates in relation to significant isolates: 75.0% of the patients recovered when rifampicin-resistant *S. aureus* was isolated and 56.1% when the isolate was sensitive to rifampicin. 75.0% of the patients recovered in the sensitive and 66.7% in the resistant group if CNS was isolated. All the three patients with MRSA infection had treatment failure whereas three out of four patients with *E. coli* and all the five patients with Cutibacterium sp. recovered. 81.8% of the patients with streptococcal infection showed recovery.

4.3. Differences between patients undergoing two-stage revision and DAIR procedure

According to our study, rifampicin-resistance of the isolated pathogen as well as patients' age, sex and diabetes mellitus had a significant effect on recovery rates in patients undergoing two-stage revision whereas BMI had no significant effect [126]. None of these factors appeared to have significant impact on the outcome among patients undergoing DAIR procedure [129].

The age-recovery curves for patients with rifampicin-sensitive and -resistant isolate in the absence and in the presence of diabetes mellitus show substantial differences between two-stage revision and DAIR procedure. Recovery rates in the rifampicin-sensitive group are high at younger ages and then show declination reaching the recovery rates of the rifampicin-resistant group, declination starts at lower age in diabetic patients and recovery rates in the resistant group are not influenced by age but are lower in diabetic patients in the two-stage revision group. On the other hand, rifampicin-resistance did not have significant effect on recovery rates, there is a slow decrease in recovery rates with advancing age in both rifampicin-sensitive and -resistant group regardless of diabetes and diabetes mellitus has limited negative effect on outcomes in both groups of patients undergoing DAIR procedure (Table 7.).

Table 7. Comparison of our findings between patients undergoing two-stage revision versus DAIR procedure

	Two-stage revision	**DAIR procedure**
Kinetics	Rapid declination at certain age	Slow linear decrease
The effect of rifampicin-resistance	Significant	Minimal
The effect of diabetes mellitus on recovery rates	Significant in the rifampicin-resistant group	Minimal
The effect of diabetes mellitus on kinetics	Declination starts at lower age	None

DOI:10.14753/SE.2024.2998

5. Discussion

5.1. Patients undergoing two-stage revision

5.1.1. Rifampicin-resistance and patient-related factors

The primary aim of our study was to determine recovery rates in patients with PJI and to investigate their correlation with rifampicin-resistance and selected patient-related factors. The overall recovery rate in the rifampicin-sensitive group was 92.5%, whereas it was as low as 60.0% if rifampicin-resistant microorganism was isolated. Various recovery rates have been reported in the literature ranging from 54.2 to 91.0% [33, 34, 130-132].

The impact of different patient-related factors has also been investigated in previous studies. High BMI (>30 kg/m^2), type 2 diabetes mellitus, chronic hypertension, rheumatoid arthritis, previous surgery, trauma and previous infection of the affected joint were associated with higher PJI incidence and significantly less favourable clinical outcomes [105, 133-138].

The vast majority of our patients were in the >60 years age group: the overall mean age was 68.6 years (standard deviation (SD) = 10.1 years). No obvious correlation between patients' age and higher incidence of PJI was found in previous studies [133, 134]. In our study, we found that age has a clinically significant impact on recovery rate in the rifampicin-sensitive but not in the -resistant group. The difference between recovery rates in the sensitive and the resistant group was more pronounced among younger patients and it was decreasing with advancing age ending up in similarly low recovery rates beyond the age of 80 years.

Diabetes mellitus has been demonstrated as a risk factor for PJIs [134, 139]. We found lower prevalence of DM in the resistant group, however, this may be due to the limited number of patients in this study. Our findings suggest negative effects of diabetes mellitus on clinical outcome: declination of recovery rates start at lower age in the rifampicin-sensitive group and reaches a lower level in diabetic patients, moreover, recovery rates are steadily lower in diabetic patients as compared to non-diabetic patients in the rifampicin-resistant group. However, further data are required to confirm

these conclusions. Nevertheless our results highlight the importance of early diagnosis and proper management and follow up of DM in patients with orthopaedic infections.

Higher incidence of PJIs in males has been demonstrated in various studies. This may be partly related to the higher number of arthroplasties performed among males than females [134, 137]. In another study, higher risk of PJI was also found in males after knee arthroplasty, however, the difference was not significant [133]. We found relevant effect of sex on recovery in our study as healing rates were significantly higher in male patients.

Correlation between higher BMI and increased PJI incidence has been previously demonstrated [135]. In our study, the average BMI was slightly higher in the resistant group (29.8 kg/m^2 [SD = 4.88kg/m^2] vs 31.4 [5.73]), however, we found no statistically significant correlation between BMI and recovery rates.

It has been confirmed that previous lesions of the joint capsule, preceding surgery and trauma of the affected joint are associated with significantly higher risk of PJI [140]. We found similar results as 100% of the patients in the resistant group and 92.6% in the sensitive group had previous surgery.

There is no obvious correlation between hypertension (HT) and the incidence of prosthetic joint infections or recovery rates in the literature [137, 141, 142]. We found high prevalence of HT both in the rifampicin-sensitive (70.4%) and in the rifampicin-resistant group (90.0%). Rheumatoid arthritis (RA) is associated with higher risk of PJI and lower recovery rates [133, 143]. Studies on the effect of stroke on recovery are lacking. Heart failure has been confirmed as an independent risk factor [141, 144]. Similarly to cardiovascular disorders, chronic pulmonary diseases (eg. COPD) have also been shown to be associated with increased risk of PJI [104, 108]. Postoperative mortality rate was found higher in patients with chronic renal failure [141].

We investigated the effect of the following risk factors on recovery rates: chronic heart disease, hypertension, chronic pulmonary disease (including COPD), chronic renal failure, ASA, rheumatoid arthritis, haematology disorders, thyroid diseases, stroke and cirrhosis. After reviewing our data, we could not draw statistically relevant conclusions due to the low number of patients in different groups with the above mentioned risk factors. Of note, this was also the case among patients undergoing DAIR procedure.

5.1.2. Microbiological background

Pathogenic spectrum has been changing worldwide: multiresistant microorganisms are becoming more prevalent in hospitalised patients including those with PJI and there is a general increase in antibiotic resistance rates [1, 145] as well as in the incidence of polymicrobial infections [146]. These factors all contribute to the emergence of difficult to treat (DTT) infections. Lower recovery rates in the rifampicin-resistant group may be explained by the limitation of treatment options when more resistant microorganisms have to be eradicated. Another aspect is that rifampicin-resistance can develop as a result of prolonged antibiotic treatment in patients with complicated infections. These include complex surgical procedures in chronic infections as well as patients with multiple comorbidities, risk factors or immunosuppression. The risk of development of rifampicin-resistance is even higher when the treatment regime is suboptimal or if there are concerns with patient's compliance.

We reviewed the microbiology reports of all patients included in our study. Fifty-three out of 73 patients (72.6%) had PJI caused by rifampicin-sensitive and 20 (27.4%) by rifampicin-resistant microorganism. We found that rifampicin-sensitive species was isolated in 80.3% among recovered patients and 33.3% in patients with treatment failure. Staphylococcus spp. were predominant in the sensitive group (66.7% of the isolates), most of which were coagulase-negative Staphylococcus spp. (27 out 36). Among CNS, the most prevalent species was *S. epidermidis* followed by *S. hominis*. Of note, *S. lugdunensis* was isolated in two cases: its importance is highlighted by the potential of this species to cause infections as severe as those caused by *S. aureus* [147]. *Cutibacterium acnes* was cultured in 8.2% of the patients. Although *C. acnes* has been recognised as a possible causative agent of PJIs with the ability of biofilm formation, it may be difficult to assess clinical significance as it can represent skin flora [49]. This Gram-positive rod is normally sensitive to most of the β-lactam antibiotics and vancomycin, however, despite of being obligate anaerobe, it is intrinsically resistant to metronidazole. An unusual pathogen, *Arthrobacter scleromae* was isolated in one case: amoxicillin, vancomycin or linezolid can be considered in the treatment of such infections, depending on antibiotic susceptibility testing results. Another challenge is the interpretation of results as there are no official breakpoints currently available. Of

note, *Streptococcus agalactiae* was the predominant streptococcal isolate in our patients (7 out of 11 cases). In one case, *S. pneumoniae* was isolated. α-haemolytic Streptococcus spp. (including *S. pneumoniae* and viridant Streptococcus spp.) require thorough antibiotic sensitivity testing especially for β-lactam antibiotics as various resistance patterns may arise possibly associated with resistance towards other classes of antibiotics, resulting in limited treatment options. *Haemophilus parainfluenzae* was found to be the causative agent in one case, highlighting the increasing clinical importance of this species often considered insignificant. Resistance to β-lactam antibiotics is determined by the combination of β-lactamase enzymes and cell wall alterations, hence careful sensitivity testing is recommended.

Gram-negative rods (including *E. coli* and other species within *Enterobacterales* order in 9 cases as well as *P. aeruginosa* in one case) and Enterococcus spp. represented the majority of isolates in the resistant group. Enterococcus spp. show increasing prevalence in the etiology of PJIs [57, 128]. In our study, 9.6% of the patients had Enterococcus spp. isolated including a(n acquired) vancomycin-resistant Enterococcus in one case. VRE represents an important infection control issue worldwide as well as a therapeutic challenge due to extremely limited treatment options. One *S. aureus* and 5 coagulase-negative Staphylococcus sp. (all of them *S. epidermidis*) were isolated. A rifampicin-resistant strain of *Corynebacterium striatum* and *Mycobacterium goodii/smegmatis* was also cultured. The clinical significance of *Corynebacterium striatum* is being recognised as it has been demonstrated to have the potential to cause a wide range of infections, mostly healthcare-associated cases. Pathogenic Corynebacteria may be sensitive to selected β-lactam antibiotics as well as clindamycin, doxycycline, sulfamethoxazole/trimethoprim and moxifloxacin, there is an increase in resistance rates towards these agents. Alternatives include vancomycin and linezolid. The treatment of *M. goodii/smegmatis* infections requires combination of antibiotics (antituberculotics) for a prolonged duration similarly to other mycobacterial infections including TB. A combination of meropenem + amikacin or sulfamethoxazole/trimethoprim + ethambutol can be considered in the initial session followed by doxycycline + ciprofloxacin.

Of note, 15.0% of the patients had previous rifampicin treatment within the resistant group and none in the sensitive group, highlighting that inappropriate antibiotic regimes can also contribute to the development of rifampicin-resistance. To prevent this,

monotherapy and functional monotherapy must be avoided. Previous rifampicin exposure carries a risk of higher incidence of rifampicin-resistant isolates even if the treatment regime was correct. Inappropriate antibiotic regimes can also contribute to the development of rifampicin-resistance. These cases include the suboptimal choice of antibiotics, route of administration, dosage and/or duration of treatment as well as the absence of outpatient follow up.

We observed the development of rifampicin-resistance in three cases of PJI: once caused by *S. aureus* (MSSA) and twice by *S. epidermidis* (both MRSE). The first patient was initially treated with intravenous cefazolin followed by oral linezolid + rifampicin and later on switched to levofloxacin + rifampicin. Both patients with MRSE infection were treated with intravenous vancomycin and oral rifampicin. However, treatment with the combination of linezolid + rifampicin and vancomycin + rifampicin has pitfalls with significant clinical relevance. Rifampicin has the ability to reduce linezolid serum concentrations resulting in lower efficacy as well as (partially) functional rifampicin monotherapy having the risk of resistance development towards rifampicin. On the other hand, vancomycin may well need a few days to reach steady-state, ie. suitable concentrations at the site of infection. However, in the first few days subtherapeutic vancomycin concentrations may lead to functional rifampicin monotherapy with the high risk of resistance development to rifampicin. After the IV session, patient was switched to PO doxycycline in one case and to PO linezolid in the other case. Of note, the combination of doxycycline and rifampicin as an oral stepdown regime has limitations too: rifampicin can also reduce the efficacy of doxycycline resulting in the same consequences as in combination with linezolid.

Linezolid is the most widely used representative of the oxazolidinone class of antibiotics having important advantages: both IV and PO formulations are available, excellent penetration to tissues even to difficult-to-reach sites, having very broad cover among Gram-positive bacteria and low level of acquired resistance [54]. For these reasons linezolid is considered as a suitable agent in the treatment of PJIs, however, it is normally reserved as a last resort option when there is no alternative choice available. On the other hand, there are some limitations of its use: it may have side effects (including serotonin syndrome and myelosuppression, therefore it is essential to monitor full blood count especially during prolonged treatment), it may be involved in drug

interactions (including rifampicin: linezolid serum concentration can be reduced potentially resulting in therapeutic failure, eg. persistence or recurrence of infection), it has no Gram-negative and anaerobic cover and it should be reserved for selected cases without other PO options as acquired resistance has already emerged, especially among Enterococcus spp. These highly resistant strains are called linezolid-resistant Enterococcus spp. (LRE) [148].

5.2. Patients undergoing DAIR procedure

5.2.1. Rifampicin-resistance and patient-related factors

The aim of the study was to determine recovery rates in patients with PJI undergoing DAIR procedure and to investigate the effect of rifampicin-resistance and selected patient-related factors on clinical outcome. The overall recovery rate in the rifampicin-sensitive group was 72.3%, whereas it was 76.9% if rifampicin-resistant microorganism was isolated. This finding is unexpected and suggests that rifampicin-resistance has no significant effect on recovery. However, this may be due to the low number of resistant isolates in the study population. Various recovery rates have been reported in the literature ranging from 54.2 to 91.0% [33, 34, 130]. It is also interesting to compare these results with patients undergoing two-stage revision due to PJI: recovery rate of 92.5% in the rifampicin-sensitive and 60.0% in the resistant group. This may suggest that rifampicin-resistance has higher impact on recovery rates among patients with two-stage revision as compared to DAIR procedure and that recovery rates were more balanced in the latter group, however, further investigations are required to confirm these findings.

The prevalence of prosthetic joint infections after knee arthroplasty was found higher among males in a study but the difference was not significant [133]. In our cohort, 59.6% were males in the sensitive and 61.5% in the resistant group. The recovery rate was 72.3% for males and 76.7% for females. We found no statistical evidence of significant impact of sex on recovery.

The majority of our patients were in the >60 years age group: the overall mean age was 68.4 years (standard deviation (SD) = 15.8 years). No relation between age and the

prevalence of PJI was found in previous studies [133, 134]. In our study, we assume that age has a limited negative impact on recovery rates both in the rifampicin-sensitive and -resistant group. However, compared to our findings among patients undergoing two-stage revision, we found no similar kinetics in the recovery-age curves in the rifampicin-sensitive group, ie. there is no significant declination after an age-threshold.

It has been shown that higher body mass index results in higher risk of PJIs [135]. In our cohort there was only a small difference of BMI between the sensitive and resistant group: 30.1 kg/m^2 and 30.6 kg/m^2. Neither clinically relevant nor statistically significant effect of BMI on recovery rates was observed in either the sensitive or the resistant group.

Diabetes mellitus has been confirmed as a significant predisposing factor for PJIs [28, 133, 134]. The prevalence of DM was found to be 5.0% in a study on patients with THR and was associated with higher rates of both surgical and non-surgical site infections [139]. In our cohort, we found higher prevalence: 16.4% of the patients had type 1 or type 2 DM. Although analysis was limited due to the low number of patients in the rifampicin-resistant group, we found a moderate but statistically not significant difference in the recovery rates of the diabetic and non-diabetic population. The characteristics of the age-recovery curves and the differences between diabetic and nondiabetic patients could not be observed as in patients undergoing two-stage revision. There was no significant difference in the recovery rates between the rifampicin-sensitive and -resistant group, regardless of diabetes mellitus. Also, the effect of DM itself was only mild on both groups. Even if not statistically significant, our data suggest the negative impact of DM on clinical outcome.

We investigated the effect of the following risk factors on recovery rates: chronic heart disease, hypertension, chronic pulmonary disease (including COPD), chronic renal failure, ASA, rheumatoid arthritis, haematology disorders, thyroid diseases, stroke and cirrhosis. After reviewing our data, we could not draw statistically relevant conclusions due to the low number of patients in different groups with the above mentioned risk factors, similarly to patients undergoing two-stage revision.

Several clinical conditions have been demonstrated to increase the risk of development of PJI, hence it is important to consider them in the management of PJIs. Factors increasing the risk of PJI after implanting primary endoprosthesis include diabetes

mellitus, urinary tract infection (UTI), high ASA score and immunosuppression [55, 149]. Another study found that obesity, COPD, excessive ethanol consumption, depression and malignancies can also be predisposing factors [56]. Uncontrolled DM, severe obesity (BMI >40 kg/m^2), liver failure, renal insufficiency, smoking, drug abuse, previous prolonged hospitalisation, malnutrition, severe acquired immunodeficiency, posttraumatic arthrosis and inflammatory arthropathy also represent risk factors for periprosthetic infections [57]. The prevalence of PJI was also shown higher among patients receiving intraarticular steroid injection [58].

63.8% of the patients had hypertension in the rifampicin-sensitive and 69.2% in the resistant group. From a different perspective, 62.0% of the patients who recovered had hypertension whereas the prevalence was 82.4% among patients with treatment failure. The prevalence was 58.8% and 60.0% in recovered patients in the sensitive and the resistant group, however, we found 76.9% and 100% of prevalence in patients who did not recover. Of note, there were only three patients in the last group. Although a previous study demonstrated that the effect of hypertension is statistically not significant [142], we found 1.3 times lower recovery rates among patients with hypertension, however, the significance of this finding is doubtful due to the low number of patients.

It has also been observed that PJIs develop more frequently in patients belonging to ASA group III and IV [150]. In the rifampicin-sensitive group we found 70.9% recovery in patients with ASA II score and 80.0% with ASA III. Recovery rates for ASA II and III patients in the resistant group were 80.0% and 50.0%. We could not confirm the negative impact of higher ASA scores on recovery rates neither in the rifampicin-sensitive nor in the rifampicin-resistant group.

Risk scores, such as KLIC and CRIME80 are used to assess the probability of therapeutic failure in patients undergoing DAIR procedure [23]. Recovery rates were compared among patients with CRIME80 score ≥3 (19 cases). Average score of recovered patients was found higher in the resistant group (2.1 vs 1.65). Distribution of patients with different scores showed similar bell curve pattern in both groups. Nineteen patients had higher than 4.5 in KLIC score. There was no significant different between the average score of recovered patients in the sensitive and the resistant group (2.91 vs 2.85). 92.3% of the patients had KLIC score in the 2-3.5 range in the resistant group.

Although the same range was predominant in the sensitive group, distribution appeared to be more balanced.

It has previously been demonstrated that the injury of knee or hip joint capsule, previous surgery and trauma significantly increase the risk of development of PJI [140]. We examined whether two-stage revision prior to DAIR had impact on recovery rates. Patients who had previous revision showed 66.7% recovery in the rifampicin-sensitive and 80.0% in the -resistant group, whereas the rates were 74.3% and 75.0% without revision. From a different approach, patients without prior revision had slightly higher recovery rates in the sensitive group, however, recovery rates were moderately higher among patients with previous revision in the resistant group. These findings indicate that previous revision had no significant effect on recovery rates in our cohort, regardless of rifampicin-resistance.

5.2.2. Microbiological background

Pathogenic spectrum has been changing worldwide. Moreover, there is a general increase in antibiotic resistance rates [1, 146] and in the incidence of polymicrobial infections [47]. The basis of the treatment of PJIs is the removal of biofilm, however, this is only achievable in the first 3-6 weeks of infection. After this period the implant most likely has to be removed resulting in significantly prolonged hospitalisation, antibiotic treatment and recovery period [49]. The biofilm grows continuously on the surface of the prosthetic material before becoming fully mature after 3-6 weeks. Postoperative exploration, effective washing, exhange of mobile components and retaining fix elements can be satisfactory for immature biofilms. However, for mature biofilms, the procedure must include complete removal of the implant [151]. Also, diffusion rate of rifampicin depends not only on the microorganism but the age of the biofilm as well. In other words, the older (more mature) the biofilm is, the more difficult to reach and maintain therapeutic concentrations of antibiotics at the site of infection.

In our study Staphylococcus spp. were predominant in the rifampicin-sensitive group 18 *S. aureus* and 18 coagulase-negative Staphylococcus spp. were isolated with the predominance of *S. epidermidis*. Of note, *S. lugdunensis* was cultured in one case. A

study in 2017 found that coagulase-negative Staphylococcus spp. are the most frequent causative agents of PJIs (30-43%) followed by *S. aureus* (12-23%) [49]. Among Streptococcus spp., *S. agalactiae* and *S. dysgalactiae* were the most predominant and *S. pneumoniae* was isolated in one case. Streptococcus spp. are the second most frequent pathogens causing PJIs, particularly *S. agalactiae*, *S. pyogenes* and *S. dysgalactiae*. A study found that 25% of the specimens grew Streptococcus sp. in relation to PJIs and blood cultures were also positive in 22% of the cases. Streptococcal PJIs most frequently develop as a result of haematogenous infection [152]. Whilst β-haemolytic Streptococcus spp. are almost exclusively sensitive to most β-lactam antibiotics, α-haemolytic Streptococcus spp. may have a more resistant profile resulting in therapeutic challenges. *C. acnes* was found to be the causative agent in six cases.

Enterobacterales (predominantly *E. coli*), *P. aeruginosa* and *E. faecalis* were the most frequent isolates in the rifampicin-resistant group. Of these microorganisms, *E. cloacae*, *K. aerogenes*, *S. marcescens* and *P. rettgeri* are chromosomal AmpC-producers. The presence of this group of enzymes results in intrinsic resistance to certain antibiotics including amoxicillin, amoxicillin/clavulanic acid and cefuroxime. However, if a derepressed mutant is present (expressing higher level of enzyme production due to various effects), the isolate is resistant to third-generation cephalosporins and piperacillin/tazobactam as well. The derepression may be a consequence of (previous) antibiotic exposure. Moreover, there is a potential for these species to express resistance to other classes of antibitiocs, including aminoglycosides (eg. gentamicin) as well as clinically relevant oral antibiotics such as fluoroquinolones (eg. ciprofloxacin) and sulfamethoxazole/trimethoprim resulting in limited therapeutic options (eg. meropenem or ertapenem). 1 *Arthrobacter polychromogenes* and 1 *Mycobacterium goodii-smegmatis* strain was also isolated. Brucella and Candida species have also been detected, however, no pathogen has been identified in 28.2% of the cases [50]. 22% of the infections were polymicrobial, mostly involving *S. aureus* [41]. We observed no development of rifampicin-resistance in our cohort and none of the patients had received previous rifampicin treatment.

MRSA infection itself is a risk factor resulting in more difficult eradication of PJIs [37]. Methicillin-resistance can be tested with cefoxitin disk or oxacillin E-test, however, it can also be detected by the PBP2a latex kit which reacts with the altered cell wall

component. This mechanism is responsible for resistance towards most of the β-lactam antibiotics. Therapeutic options can be further limited by the concomitant presence of resistance to other classes of antibiotics. Possible therapeutic options include clindamycin, doxycycline, sulfamethoxazole/trimethoprim, vancomycin and linezolid.

One study concluded that in case of PJIs caused by low-virulence microorganisms (eg. coagulase-negative Staphylococcus spp., Cutibacterium spp. and Acinetobacter spp.) DAIR procedure can be appropriate and result in full recovery, however, this finding should be treated with caution [41]. The incidence of PJIs caused by CNS is worldwide increasing [153]. In our cohort, 33.3% of the patients in the sensitive group had infection caused by coagulase-negative Staphylococcus sp., higher than indicated in the literature, however, no infections due to CNS was found in the resistant group. The prevalence of Enterococcus spp. in orthopaedic infections is also on an upward trend [57]. In our cohort, 7.5% of the patients had enterococcal PJI, all of them caused by *Enterococcus faecalis*.

Antibiotic regimes were also reviewed in our study. Rifampicin administration in combination was documented in 34 cases, however, rifampicin was used in monotherapy in two patients. When comparing antibiotic regimes, ciprofloxacin was the combination partner when the highest number of rifampicin-resistant microorganisms were isolated (30.8%). This may be partly due to the aim to provide Gram-negative cover, however, another aspect should also be considered. Among fluoroquinolones, ciprofloxacin has only moderate activity against Gram-positive bacteria whereas levofloxacin and especially moxifloxacin have significantly more pronounced Gram-positive cover. Combination with rifampicin has the potential to result in functional rifampicin monotherapy that may rapidly lead to resistance. The most frequent combinations in the sensitive group were sulfamethoxasole/trimethoprim + rifampicin (19.1%) and levofloxacin + rifampicin (17.0%). These combinations are considered adequate as both sulfamethoxasole/trimethoprim and levofloxacin have clinically reliable activity against Gram-positive bacteria.

5.3. Differences between patients undergoing two-stage revision and DAIR procedure

Rifampicin-resistance of the isolated pathogen as well as patients' age, sex and diabetes mellitus had a significant effect on recovery rates in patients undergoing two-stage revision whereas BMI did not. None of these factors appeared to have significant impact on outcome among patients undergoing DAIR procedure.

The age-recovery curves for patients with rifampicin-sensitive and -resistant isolate in the absence and in the presence of diabetes mellitus show substantial differences between patients with two-stage revision and DAIR procedure. In patients undergoing two-stage revision, recovery rates in the rifampicin-sensitive group are high at younger ages and then show declination reaching the recovery rates of the rifampicin-resistant group. Declination begins at lower age in diabetic patients and recovery rates in the resistant group are not influenced by age, however, they are lower in diabetic patients. On the other hand, rifampicin-resistance did not have significant effect on recovery rates: there is a slow decrease in recovery rates with advancing age in both rifampicin-sensitive and -resistant group regardless of diabetes and diabetes mellitus has limited negative effect on outcomes in patients undergoing DAIR procedure. The reasons of the above differences are unclear, indicating the need for further investigations.

As a closing remark, a few limitations of these studies need to be considered. Relatively few patients were included, making statistical analysis and determining significance challenging in certain cases. Our study cohorts included patients with PJI affecting different joints, which could also interfere with our results. However, identical therapeutic procedures were performed in all cases with two-stage revision, therefore in our opinion this factor might only have moderate effect on the final conclusions. Also, the surgical technique of DAIR was not excactly the same for all patients as mobile parts were not always exchanged, therefore this variable was also evaluated in our study.

DOI:10.14753/SE.2024.2998

6. Conclusions

We investigated the effect of rifampicin-resistance and various factors on the recovery rates of patients with prosthetic joint infections in two groups: patients undergoing two-stage revision and patients with DAIR procedure.

6.1. Patients undergoing two-stage revision

1., Rifampicin-resistance, age, sex and type 2 diabetes mellitus had significant impact on recovery rates but not the BMI. Correlation between age and recovery was seen in the rifampicin-sensitive group with a rapid declination after the age of 70 years. No such correlation was observed in the resistant group. In diabetic patients, we found similar trends with some marked differences: the declination of recovery probability begins earlier, after the age of 60 years and the recovery rates in the resistant group are significantly lower than in the non-diabetic population.
2., Majority of the patients had PJI caused by rifampicin-sensitive microorganism and rifampicin-resistance was associated with significantly lower recovery rates. The isolation of rifampicin-sensitive microorganism was notably more frequent among recovered patients than in patients with treatment failure.
3., The most frequent isolates were coagulase-negative Staphylococcus spp. in the sensitive group, whereas Enterococcus spp. and Gram-negative rods were predominant in the resistant group. Polymicrobial infections were also identified.
4., Previous rifampicin treatment was seen only in the resistant group and development of rifampicin-resistance was observed in three cases of staphylococcal infections.

6.2. Patients undergoing DAIR procedure

1., Rifampicin-resistance, BMI and sex had no statistically significant impact on recovery rates, however, increasing age and diabetes may have a negative clinical impact on clinical outcome.
2., The majority of the patients had PJI caused by rifampicin-sensitive microorganism, however, in our study we could not find enough evidence to confirm that rifampicin-

resistance is associated with lower recovery rates. The isolation of rifampicin-sensitive microorganisms was not more frequent among recovered patients. Of note, only Gram-positive species were observed in the treatment failure group with the majority of Staphylococcus spp.

3., The most frequent isolates were Staphylococcus spp. in the sensitive and Gram-negative rods in the resistant group. Polymicrobial infections were also identified.

4., No development of rifampicin-resistance was observed in the study population.

6.3. Clinical relevance

1., Recognition of microbiological and patient-related factors may help estimate and reduce treatment failure rates after two-stage revision surgery and DAIR procedure performed in patients with PJI. This approach is extremely useful for patients during the follow up period after surgery, however, it can also help finding the most appropriate procedure on an individual basis. The probability of recovery can also be improved by identifying relevant factors and influence them accordingly if feasible.

2., The significance of the isolated microorganism(s) should always be carefully assessed as it may prove difficult to distinguish true pathogens from colonisers and contaminants. Even skin flora members can also cause PJIs, especially in chronic cases, eg. coagulase-negative Staphylococcus spp., Corynebacterium spp. and Cutibacterium spp.

3., When comparing antibiotic regimes, ciprofloxacin was the combination partner when the highest number of rifampicin-resistant microorganisms were isolated. In order to prevent the development of rifampicin-resistance, monotherapy and functional monotherapy should be avoided, appropriate combinations should be used (with suitable activity against Gram-positive bacteria of the combination partner) and delayed administration of rifampicin may be required in selected cases. Possible drug interactions should also be taken into account.

4., It is interesting to compare our findings between patients undergoing two-stage revision and DAIR procedure. The reasons of these findings and their clinical relevance are yet to be investigated.

DOI:10.14753/SE.2024.2998

7. Summary

Rifampicin plays a key role in the management of prosthetic joint infections (PJIs), however, the emergence of rifampicin-resistance is associated with less favorable clinical outcomes. Patient-specific data, comorbidities and the antibiotic resistance of microbiological isolates were collected and reviewed. Obtained data were statistically analysed with a logistic regression model. The first aim of our study was to investigate the impact of rifampicin-resistance and other patient-related factors on recovery rates among patients with PJI undergoing two-stage revision. We reviewed medical records and microbiology reports of 73 patients undergoing two-stage revision due to PJI between 2017 and 2019. Rifampicin-sensitive microorganism was isolated in 53 cases (72.6%). Recovery rate was 92.5% in the sensitive and 60.0% in the resistant group. In the rifampicin-sensitive group, the probability of recovery decreased with advancing age with a significant drop above the age of 60 years. The effect of age is negligible in the rifampicin-resistant group. We also found that type 2 diabetes mellitus has a negative effect on recovery whereas higher recovery was observed among males. Coagulase-negative Staphylococcus spp. were predominant in the rifampicin-sensitive (50.0% of the isolates) and Gram-negative rods in the resistant group (40.0%). The second aim of our study was to investigate the impact of rifampicin-resistance and other patient-related factors on recovery rates among patients with PJI undergoing DAIR (Debridement, Antibiotics and Implant Retention) procedure. We collected and reviewed medical records and microbiology reports of 67 patients undergoing DAIR due to PJI between 2014 and 2021. Rifampicin-sensitive microorganism was isolated in 47 cases. Recovery rate was 72.3% in the sensitive and 76.9% in the resistant group. Based on our results, higher age and diabetes mellitus may have a clinically relevant negative impact on clinical outcome, however, this effect was not statistically significant. We observed no clinically relevant effect of rifampicin-resistance, sex and body mass index (BMI) on recovery rates among patients undergoing DAIR due to PJI. *Staphylococcus aureus* and coagulase-negative Staphylococcus spp. were predominant in the rifampicin-sensitive (66.6% of the isolates) and Gram-negative rods in the resistant group (65.2%).

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DOI:10.14753/SE.2024.2998

9. Bibliography of the candidate's publications

Publications related to current PhD-thesis:

Krizsán G, Sallai I, Veres D S, Prinz Gy, Szekér D, Skaliczki G.
Rifampicin resistance and risk factors associated with significantly lower recovery rates after two-stage revision in patients with prosthetic joint infection
Journal of Global Antimicrobial Resistance, 2022;30:231-236.
PMID: 35764215, doi: 10.1016/j.jgar.2022.06.020.

Krizsán G, Sallai I, Veres D S, Prinz Gy, Kovács M, Skaliczki G.
Investigation of the effect of rifampicin resistance and risk factors on recovery rates after DAIR procedure in patients with prosthetic joint infection
Journal of Orthopaedic Surgery and Research, 2023;18(1):611.
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DOI:10.14753/SE.2024.2998

10. Acknowledgements

First I would like to express my gratitude to my tutor, **Gábor Skaliczki MD, PhD** for all his support and advices throughout my work and that I have finally found a so very helpful tutor after such a long search.

I am thankful for my previous student in Medical Microbiology and now specialist in Orthopaedics, **Imre Sallai MD** for all his clinical advices.

I am thankful to **Dániel Sándor Veres PhD** for all his work and advices in statistical analysis of clinical data.

I am thankful to **Donát Szekér MD** for his contribution to data collection of patients undergoing two-stage revision.

I am thankful to **Máté Kovács MD** for his contribution to data collection of patients undergoing DAIR procedure.

I am thankful to **Andor Marencsák** and **Brigitta Firnigel** for their help in the collection and statistical analysis of clinical data.

I am thankful to **Csaba L. Maródi MD**, Consultant Microbiologist who revealed the beauty, complexity and importance of Medical Microbiology at our 3rd year seminars in the university and who is still a great friend and colleague.

I am thankful to **Michael J. Weinbren MD**, Consultant Microbiologist who gave me so much help and support in my first Consultant Microbiologist role in England, in Chesterfield which was the beginning of a great journey in 2014.

I am thankful to **László Tretter MD, PhD**, Professor of Biochemistry for all his support and teaching during my former research works in biochemistry.

I am thankful to my great secondary school teachers and Piarist fathers, **Rudolf Urbanek**, my forever master in biology and **Lajos Dragon Faragó**, my forever master in chemistry who taught me so much in biology and chemistry, giving life-long knowledge and experience.

I am thankful to **my true friends** who supported me with their presence, advices or simply by understanding that I was sometimes unable to meet them due to my duties.

DOI:10.14753/SE.2024.2998

I am thankful to **my mother** for all her help and kindness.

I am thankful to my youngest brother, **Dániel** who is one of the very few fellows who understands me and my true intentions.

I am especially and forever grateful to my beloved grandparents, **Lajos Andor Kozma LLD** and **Mária Kapcsos** who are already looking after us from the celestial heights. Their life is the true example for me. Their love, support and understanding, "being always there", whenever needed, made me become who I am today. Without them I would have been lost.

But first of all I am truly grateful to my family: my beloved wife, **Ágnes** and daughter, **Eszter Emma** who always stand with me, understand me, support me and make me happy. Their love, empathy, patience and joy helped me so much when writing my PhD thesis and help in my daily life too. Without them I would be lost.