

# BMJ Open Treatment of periprosthetic joint infections guided by minimum biofilm eradication concentration (MBEC) in addition to minimum inhibitory concentration (MIC): protocol for a prospective randomised clinical trial

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## ABSTRACT

**Introduction** Prosthetic joint infections (PJIs) are disastrous complications for patients and costly for healthcare organisations. They may promote bacterial resistance due to the extensive antibiotic use necessary in the PJI treatment. The PJI incidence is estimated to be 1%–3%, but the absolute numbers worldwide are high and increasing as large joint arthroplasties are performed by the millions each year. Current treatment algorithms, based on implant preserving surgery or full revision followed by a semitailored antibiotic regimen for no less than 2–3 months, lead to infection resolution in approximately 60% and 90%, respectively. Antibiotic choice is currently guided by minimum inhibitory concentrations (MICs) of free-living bacteria and not of bacteria in biofilm growth mode. Biofilm assays with relatively rapid output for the determination of minimum biofilm eradication concentrations (MBECs) have previously been developed but their clinical usefulness have not been established.

**Methods and analysis** This single-blinded, two-arm randomised study of hip or knee staphylococcal PJI will evaluate 6-week standard of care (MIC guided), or an alternative antibiotic regimen according to an MBEC-guided-based decision algorithm. Sixty-four patients with a first-time PJI treated according to the debridement, antibiotics, and implant retention principle will be enrolled at a single tertiary orthopaedic centre (Sahlgrenska University Hospital). Patients will receive 14 days of standard parenteral antibiotics before entering the comparative study arms. The primary outcome measurement is the proportion of changes in antimicrobial regimen from first-line treatment dependent on randomisation arm. Secondary endpoints are unresolved infection, how microbial properties including biofilm abilities and emerging antimicrobial resistance correlate to infection outcomes, patient reported outcomes and costs with a 12-month follow-up.

**Ethics and dissemination** Approval is received from the Swedish Ethical Review Authority, no 2020-01471 and the Swedish Medical Products Agency, EudraCT, no 2020-003444-80.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is a translational randomised controlled trial with a sufficiently large patient number to assess if minimum biofilm eradication concentration (MBEC) diagnostics significantly changes antibiotic choice compared with minimum inhibitory concentration (MIC) diagnostics in prosthetic joint infection (PJI).
- ⇒ Using a multidisciplinary approach, this prospective study addresses the important issue of biofilm in implant preserving PJI treatments.
- ⇒ This study allows a high degree of standardisation, since patients are admitted to a single-centre unit dedicated to orthopaedic infections.
- ⇒ The study endpoints are of high clinical and microbiological relevance, that is, reduced antibiotic use, bacterial biofilm properties and emerging antibiotic resistance in recurrent infections.
- ⇒ A limitation of this study is that it is underpowered regarding the comparison of infection resolution in MIC and MBEC groups.

**Trial registration number** ClinicalTrials.gov ID: NCT04488458.

## INTRODUCTION

### Background

Implant-associated infections and the global rise of antibiotic resistance, to which treatment of the former contributes, lead to increased morbidity, mortality and high medical costs.<sup>1 2</sup> Implant-associated infections will almost uniformly involve biofilm formation.<sup>3 4</sup> In fact, approximately 70% of hospital-acquired infections are associated with medical implants and caused by staphylococcal biofilms.<sup>5</sup> Biofilms can be described as a community of bacterial cells interconnected by their protective extracellular

matrix and pheromone-based signalling.<sup>6–8</sup> Although a few antibiotics have moderate antibiofilm properties,<sup>9</sup> most are developed to combat acute infections caused by free-living planktonic bacterial cells. Therefore, resolving treatment challenges<sup>10</sup> and overcoming antibiotic resistance in clinical biofilms<sup>11</sup> constitutes a paradigm shift. Biofilms may mount a 1000-fold increase in resistance to antibiotics, compared with planktonic bacteria.<sup>12</sup> The proximity between cells and other molecular mechanisms in the biofilm community facilitates horizontal transfer of antimicrobial resistance genes.<sup>13–15</sup> In addition, the level of antimicrobial resistance in bacteria causing implant-associated infections has increased worldwide, leaving patients with fewer treatment options.<sup>16,17</sup> Finally, the high consumption of resistance-driving antibiotics (eg, cephalosporins and fluoroquinolones) necessary to overcome implant-associated infections adds to the global overuse of antibiotics, therefore efforts in optimising diagnostics and gathering an evidence base for shortened therapy cannot wait.

Worldwide, millions of hip and knee replacements are performed annually and the number is projected to rise.<sup>18–20</sup> In Sweden, the current combined figure is approximately 36 000.<sup>21,22</sup> Considering conservative prosthetic joint infection (PJI) incidence values of 0.69%–1.38%,<sup>23</sup> the burden for society is high, in terms of UK hospital costs about £33 000/infection.<sup>24</sup> Although a consensus definition of successful outcomes is lacking,<sup>25</sup> overall treatment success rates for PJI are between 65% and 90%,<sup>26–31</sup> mainly depending on the causative microbe and the clinical presentation (early postsurgical, chronic or late acute PJI) in relation to the selected treatment regimen. Medicosurgical approaches involve radical debridement, staged revision surgery or infrequently implant removal, combined with long-term antibiotics. In the clinical workup, the biofilm-forming ability of the causative pathogen is almost never investigated, and the *in vitro* antimicrobial susceptibility testing is based on planktonic bacterial cultures (minimum inhibitory concentrations (MICs)). Hence, there is a mismatch between the well-established conceptual role of biofilm in implant-related infections and the diagnostic method to guide the choice of antimicrobials. *In vitro* biofilm susceptibility measurements are empirical assumptions of antibiotic activity, since the *in vivo* biofilm may differ in structure, metabolism, dynamics, as well as antimicrobial biofilm penetration and activity.<sup>32</sup> To our knowledge, no randomised controlled trials have been undertaken to evaluate the clinical usefulness of determining minimum biofilm eradication concentration (MBEC) to guide the treatment of biomaterial-associated infections. A few studies in cystic fibrosis patients suffering recurrent *Pseudomonas aeruginosa* infections were unable to indicate superiority of MBEC-guided treatment,<sup>33</sup> but the results are not inferable (eg, chronic infections caused by mature non-staphylococcal biofilms subjected to antimicrobial agents with other mode of actions). As there is a paucity of direct evidence supporting that standardised biofilm

susceptibility testing would improve patient outcome,<sup>32</sup> clinical studies should be carefully designed to stepwise investigate if there is a useful relationship between *in vitro* MBEC and therapeutic choices. This is reflected in the main objectives of this study.

### Study objectives

Primary: to assess the proportions of antimicrobial regimens other than standard of care (SOC) that will be administered following guidance by MBEC and MIC testing combined compared with MIC testing alone.

Secondary: (1) to evaluate how MBEC-guided antibiotic regimens affect: (a) infection resolution, (b) patient-related outcome measures (PROMs), (c) drug tolerability and (d) resistance development of relapse strains and (2) to determine the virulence properties of clinical strains of *Staphylococcus* spp isolated from PJI (eg, biofilm formation ability, biofilm antimicrobial resistance and carriage of virulence genes).

### Rational for comparator choice

The cumulative evidence supports the use of certain antimicrobial agents (eg, rifampicin (RIF) and levofloxacin (LEVO)) for implant preserving staphylococcal biofilm infections,<sup>29</sup> despite the equal *in vitro* potency of many other compounds against the same bacterial strain grown planktonically. Although the difference in efficacy cannot merely be explained by tissue penetration, comparator compounds must have similar bioavailabilities in addition to *in vitro* MIC–MBEC variability. Since the first-line SOC consist of a combination (RIF+LEVO) and fusidate (FUS) also require a companion antibiotic, the comparator allocated is also set as a two-drug combination. All study antibiotics have, to varying extent, previously been used as companion drugs to RIF, although degrees of interaction and added clinical effect thereof is insufficiently known. The variable risk of antagonism demonstrated *in vitro*<sup>34</sup> for certain combinations is not substantiated *in vivo*.<sup>35</sup> To compensate for decreased exposure following comedication with RIF, dosing of several companion drugs is therefore moderately above standard including trimethoprim/sulfamethoxazole (SXT), LEVO and clindamycin. The study design only admits early postoperative or acute haematogenous PJI treated according to debridement, antibiotics and implant retention (DAIR). This mirrors the clinical circumstances in which a higher risk for failure compared with revisions is accepted, to possibly spare the patients from further treatment related morbidity and mortality<sup>36</sup> and reduce costs. Decreasing the risk of failure following DAIR is evidently much desired. Overall, the bacteriological aetiology of infections treated by DAIR is more easily determined due to the shorter culture times compared with chronic low-grade infections,<sup>37</sup> making DAIR-treated patients suitable for this study design.

### Trial design

This is a phase IV, 1:1 randomised, single-blinded, parallel-group, exploratory study. Patients, patient interest groups

**Table 1** Criteria to be met for study participation

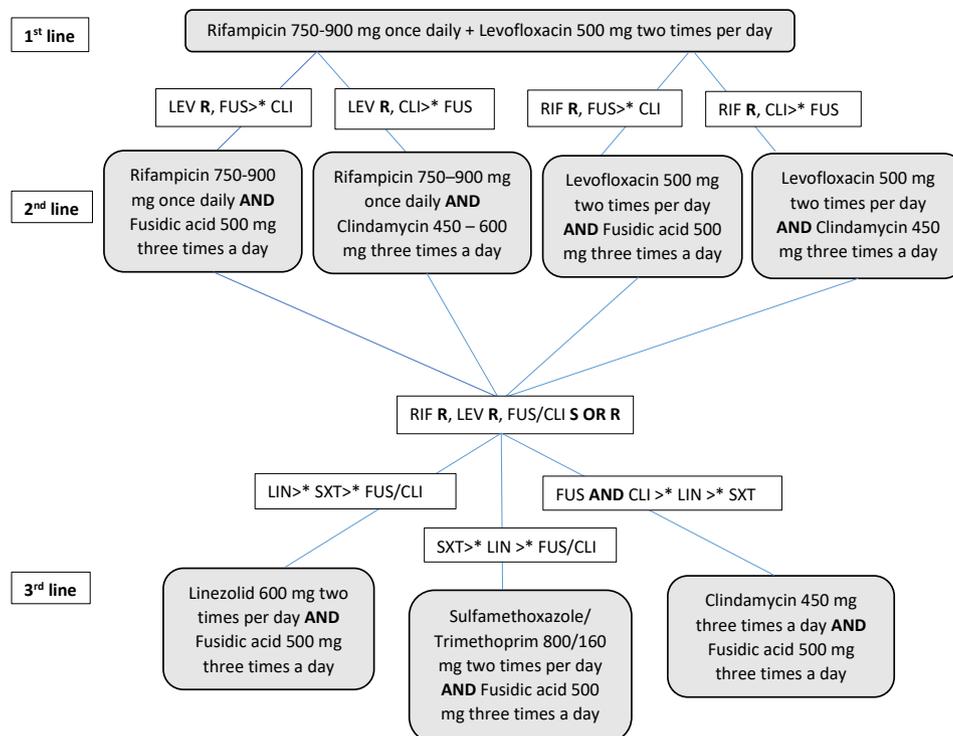
Inclusion criteria	Exclusion criteria
First-time prosthetic joint infection in hip or knee according to the Musculoskeletal Infection Society definitions <sup>47</sup>	Allergy, previous toxic event or unacceptable drug interaction to most effective antibiotic combination according to either minimum inhibitory concentration or MBEC
First debridement, antibiotics and implant retention	Severe drug interactions to MBEC-guided compound retention
Monomicrobial staphylococcal infection	Pregnancy and women of childbearing potential
14 days of intravenous treatment with either cloxacillin or vancomycin	
MBEC, minimum biofilm eradication concentration.	

or the public were not involved in the design or dissemination plans of the present research.

**METHODS AND ANALYSIS**  
**Inclusion and randomisation**

All patients meeting the inclusion criteria (table 1) will be consecutively enrolled at the Unit for Orthopaedic Infections (UOI), which is part of a large tertiary orthopaedic centre at Sahlgrenska University Hospital (Möln dal, Sweden). The UOI is dedicated to optimising the management of orthopaedic infections. In case of inadequate enrolment, a secondary medium-sized orthopaedic unit (previous collaborations) will be engaged.

Patient consent acquisitions and consecutive 1:1 randomisation using eight blocks of eight envelopes will be carried out during postoperative hospitalisation once routine bacteriological diagnostics are consistent with a monomicrobial aerobic staphylococcal infection, which is defined as no growth of a second microorganism in two or more cultures within 7 days. Randomisation allocates



**Figure 1** Decision tree on antibiotic combinations other than standard of care in the comparator study arm. MBEC threshold for substituting with second-line or when applicable third-line antibiotic combinations; RIF: MBEC/MIC>8, LEVO: MBEC/MIC>5, FUS: MBEC/MIC>3, CLI: MBEC/MIC>4, LZD: MBEC/MIC>2, SXT: MBEC>MIC. \*Interpreted as more effective than. CLI, clindamycin; FUS, fusidate; LEVO, levofloxacin; LZD, linezolid; MBEC, minimum biofilm eradication concentration; MIC, minimum inhibitory concentration; RIF, rifampin; SXT, trimethoprim/sulfamethoxazole.

**Table 2** Intraoperative sampling protocol

Timing	Sample number and size	Sampling procedure	Sample type	Transport conditions
Directly on opening of the joint	5 pieces of 1–1.5 cm <sup>3</sup> each	Interspaced biopsies with 5 separate sterile instruments	Macroscopically inflamed periprosthetic tissues at the implant–bone interface	5 separate sterile containers for each sample transported the day of surgery

to an SOC arm guided by MIC, or a comparative arm where antibiotic combinations will be decided through an MIC+MBEC algorithm (figure 1). First-line SOC is always RIF+LEVO. If resistance to first-line antibiotics (also RIF + LEVO) in the MIC arm patients will receive a second-line or third-line combination<sup>35</sup> according to the same hierarchy as for MBEC in the decision tree (figure 1). The hypothesis is that the addition of MBEC susceptibility testing will potentially guide towards a different treatment than if only MIC susceptibility testing is used. For all administered antimicrobial agents, staphylococcal strains must be susceptible using disk diffusion and MIC susceptibility testing according to the EUCAST SIR classification,<sup>38</sup> regardless of MBEC level.

### Intervention

The surgical protocol consists of standardised DAIR surgery, consisting of thorough debridement, irrigation and exchange of plastic inserts and modular components, with no major differences from best practice DAIR recommendations.<sup>31</sup> Procedures will only be performed by experienced arthroplasty surgeons. In each patient, periprosthetic tissue samples for microbiological culturing are obtained in a standardised manner (table 2) and transported to the clinical laboratory the same day in empty sterile containers.

Before entering either study arm, patients will receive 14 days of parenteral antibiotics, cloxacillin (2g four times a day) for methicillin sensitive staphylococci or vancomycin (trough concentration 15–20 mg/L) for methicillin resistant staphylococci or in cases of type 1 penicillin allergy. The main reasons for randomising patients following parenteral antibiotics are: (1) sample size calculation is based on resistance to SOC with biofilm efficacy, which is not a feature of neither cloxacillin nor vancomycin and (2) cloxacillin resistance is high among many coagulase negative staphylococci (CoNS), while vancomycin susceptibility is almost present, which would skew allocations to parenteral antibiotics. Antibiotic combinations will be selected from six non-cell wall active antistaphylococcal antibiotics with high peroral bioavailabilities, acceptable bone penetration and established clinical breakpoints (EUCAST) commonly used in the treatment of PJIs (figure 1). Subjects in both arms will go through a 6-week antibiotic regimen and are expected to participate in the study through the 12-month follow-up period for relapse or reinfection. In the event of short-term gastrointestinal intolerance to RIF (addressed by stepwise reintroduction and subsequent tolerability), self-limiting

infectious gastroenteritis or similar non-severe interruptions, the duration of the peroral phase will be adjusted accordingly, but the actual number of weeks on full dose antibiotics will not exceed 6 weeks. Reinfection is defined as a consecutive infection caused by a different bacterial strain. Relapse is defined as a reemerging infection caused by the same bacterial strain (identical species and antibiogram) due to unsuccessful treatment. The first-line SOC treatment is RIF and LEVO, and the comparator treatments will be a combination of two of the following antimicrobial agents: clindamycin, FUS, LEVO, linezolid, RIF or SXT. In cases of reinfection or relapse, new tissue sampling and culturing will be performed to confirm the infection diagnosis, additional MIC and MBEC susceptibility testing, and the patient will be treated according to SOC if applicable.

Routine diagnostics for orthopaedic implant infections will be carried out at the clinical microbiology lab, Sahlgrenska University Hospital (SWEDAC no. 1240). In brief, under sterile conditions each of the five tissue samples (1–1.5 cm<sup>3</sup>) is sliced into smaller tissue pieces which are smeared and cultured on five horse-blood (5%) Columbia agar plates and chocolate agar plates and inoculated into enrichment thioglycolate broth (media department, clinical microbiology lab, Sahlgrenska University Hospital). When there is evidence of a monomicrobial staphylococcal infection (see table 1 for inclusion criteria), MIC determination for all study antibiotics is undertaken utilising a custom-made microbroth dilution (MBD) plate including increasing double concentrations of nine antimicrobial agents commonly used in the treatment of staphylococcal PJI (figure 2). The MBD plate has been formulated and validated for this study (Sensititre Custom AST Plate, Plate code: SWE1GOTH, Thermo Fisher Scientific, UK). Subcultured strains are transferred, no later than day 7 after biopsy sample culturing, to the laboratory at the department of biomaterials (University of Gothenburg) for biofilm culturing and MBEC determination using the same MBD plate.

### Determining MIC, MBEC and quantifying biofilm biomass

Standard MIC susceptibility testing is performed from planktonic cultures from each clinical strain according to EUCAST recommendations.<sup>38</sup> In brief, the clinical strain is subcultured on 5% horse-blood Columbia agar plates (media department) and incubated overnight (o.n.) at 37°C. The isolated colonies are added to 2 mL of PBS until a 0.5 (0.4–0.6) McFarland turbidity standard is reached, equivalent to 1.5×10<sup>8</sup> CFU/mL, using a densitometer

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG	RIF 0.015	RIF 4	LEVO 0.06	LEVO 16	FUS 8	CLI 0.5	CLI 128	LZD 128	OXA+ 0.5	VAN 0.5	VAN 128
B	NEG	RIF 0.03	RIF 8	LEVO 0.12	LEVO 32	FUS 16	CLI 1	CLI 256	SXT 1/19	OXA+ 1	VAN 1	FOX 1
C	POS	RIF 0.06	RIF 16	LEVO 0.25	LEVO 64	FUS 32	CLI 2	LZD 2	SXT 2/38	OXA+ 2	VAN 2	FOX 2
D	POS	RIF 0.12	RIF 32	LEVO 0.5	LEVO 128	FUS 64	CLI 4	LZD 4	SXT 4/76	OXA+ 4	VAN 4	FOX 4
E		RIF 0.25	RIF 64	LEVO 1	FUS 0.5	FUS 128	CLI 8	LZD 8	SXT 8/152	OXA+ 8	VAN 8	FOX 8
F		RIF 0.5	RIF 128	LEVO 2	FUS 1	FUS 256	CLI 16	LZD 16	SXT 16/304	OXA+ 16	VAN 16	FOX 16
G		RIF 1	LEVO 0.015	LEVO 4	FUS 2	CLI 0.12	CLI 32	LZD 32	SXT 32/608	OXA+ 32	VAN 32	FOX 32
H		RIF 2	LEVO 0.03	LEVO 8	FUS 4	CLI 0.25	CLI 64	LZD 64	SXT 64/1216	OXA+ 64	VAN 64	FOX 64

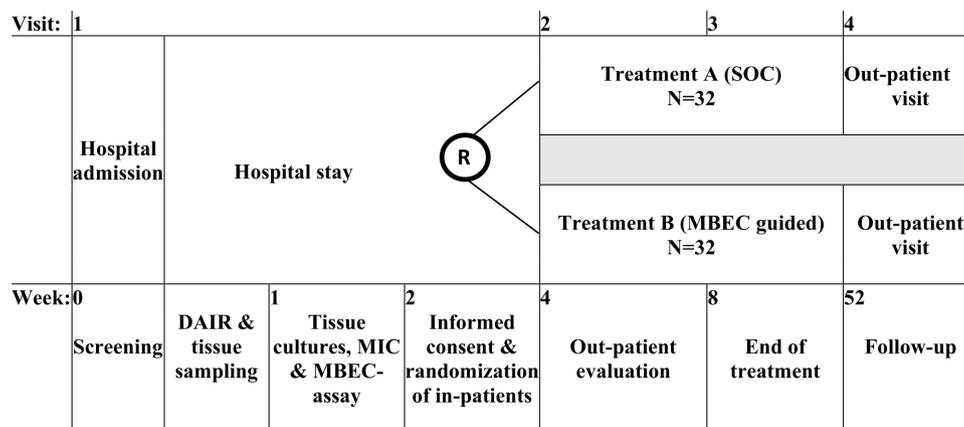
**Figure 2** Representation of the custom-made microbroth dilution plate for the determination of minimum inhibitory concentration and minimum biofilm eradication concentration. The susceptibility plate contains nine antimicrobial agents in doubling increasing concentrations (mg/L): levofloxacin (LEVO), clindamycin (CLI), oxacillin+2% NaCl (OXA+), fusidate (FUS), linezolid (LZD), rifampin (RIF), trimethoprim/sulfamethoxazole (SXT), vancomycin (VAN) and ceftiofuran (FOX). In the first column, two wells for positive and negative controls are included, and the last four wells are empty since these pegs containing biofilms are used for colony-forming unit counting.

(DensiCHECK, Bioré). Then, 50  $\mu$ L is added to 11.5 mL of Mueller-Hinton Broth 2 (MHB, Sigma Aldrich, Missouri, USA). A volume of 100  $\mu$ L is added to each well of the MBD plate, reaching a final inoculum concentration of  $5 \times 10^5$  CFU/mL in the well. The plate is incubated at 37°C for 20 hours and then read by ocular inspection and compared with the established breakpoints for staphylococci by EUCAST<sup>38</sup> to determine if the strain is susceptible (S), susceptible increased exposure (I) or resistant (R). The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye.<sup>39</sup> The *Staphylococcus aureus* ATCC 29213 strain is used as control.

The Calgary Biofilm Device (CBD, MBEC Assay, Innovotech, Edmonton, Canada)<sup>40</sup> allows for the reproducible in vitro determination of biofilm antibiotic susceptibilities. Zaborowska *et al* combined the CBD and a custom made MBD antibiotic plate as a diagnostic tool for MBEC testing of clinical strains from orthopaedic implant-related infections and was further evaluated by Svensson Malchau *et al* in a retrospective study using a relatively large collection of staphylococci from PJI.<sup>4 41</sup> In this study, MBEC determination is performed as previously described.<sup>4 40 42</sup> The clinical strain is subcultured on blood agar plates and incubated o.n. at 37°C. Isolated colonies are added to 4 mL MHB until an  $OD_{546nm} = 0.13$  or 0.27 for *S. aureus* or CoNS, respectively, is reached, which corresponds to  $10^8$  CFU/mL. This OD suspension is then diluted 10 times and a 150  $\mu$ L volume is added into each

well of the 96-well CBD (final inoculum of  $5 \times 10^5$  CFU/mL in the well) and incubated at 37°C and 125 rpm under humid conditions for 24 hours, for the formation of biofilms on the lid pegs. The next day, the peg lid of the CBD containing the biofilms is rinsed in a new 96 microtiter well plate containing 200  $\mu$ L 0.9% saline. To quantify the number of viable counts in the biofilms (CFU/peg), three pegs are removed with sterilised flamed pliers, each placed in 1000  $\mu$ L saline, sonicated for 1 min (40 Hz), vortexed at high speed for 1 min and a volume of 20  $\mu$ L from a series of 10-fold serial dilutions (in 0.9% saline+0.1% triton X) is cultured on blood agar plates at 37°C o.n. To determine the MBEC for all antimicrobial agents, the biofilm peg lid is placed in the MBD plate, previously reconstituted with 100  $\mu$ L MHB, and incubated at 37°C for 20 hours. Each peg lid is then rinsed two times in saline for 1 min, placed in a neutralising recovery plate containing 200  $\mu$ L MHB supplemented with 20.0 g/L saponin, 10 g/L Tween-80, 1.25 g/L L-histidine, 1.25 g/L L-cysteine and 2.5 g/L reduced glutathione, sonicated for 1 min to detach the biofilm into each well, and incubated o.n. at 37°C. MBEC is determined by ocular inspection using the Sensititre Manual Viewbox and defined as the minimum dose of the antimicrobial agent without visible growth or turbidity. In case of bacterial growth after exposure to the highest antimicrobial concentration, the subsequent doubling concentration is chosen as MBEC value. The time required from subculturing the strain to the final MBEC results is 5 days.

The degree to which the strains form biofilm is assessed by the microtiter plate test using crystal violet (CV) staining, which method has been previously described.<sup>41 43</sup> In short, one colony from each strain is inoculated in tryptic soy broth (TSB) (Scharlau, Barcelona, Spain) (+0.25% glucose for *S. aureus*) and cultured o.n. at 37°C and 200 rpm. The cell suspension is adjusted to  $OD_{546} = 1$ , diluted 1:40 in TSB (+ glucose for *S. aureus*) and 200  $\mu$ L are added in triplicates to 96-well polystyrene microtiter plates (BioLite Cell Culture Treated Plates, Thermo Scientific, Massachusetts, USA) and incubated for 24 hours at 37°C. Plates are rinsed 3 times by immersion in water, stained with 200  $\mu$ L CV (2%) (VWR, Pennsylvania, USA) for 5 min, rinsed 3 times and air dried. The stain is eluted in 200  $\mu$ L ethanol-acetone (80:20, vol/vol) for 5 min, and 150  $\mu$ L are transferred to a new plate to determine the absorbance at 595 nm using a plate reader (FLUOstar Omega, BMG Labtech, Offenburg, Germany). Three wells contain sterile TSB serve as blanks. The strain's biofilm-forming ability is categorised according to Baldassarri *et al* breakpoints.<sup>44</sup> Whole genome sequencing will be performed in all clinical staphylococcal isolates to type the strains and detect the carriage of virulence and antibiotic resistance genes. The bacterial strains are stored at -80°C for as long as adequate storage quality and space can be secured.



**Figure 3** Schematic summary and timeline of the study. Informed consent will be obtained and block randomisations undertaken during the postoperative hospital stay. The oral antibiotic regimen will take 6 weeks, and the patient will be followed up to 1 year postoperatively for relapse or reinfection events. DAIR, debridement, antibiotics and implant retention; MBEC, minimum biofilm eradication concentration; MIC, minimum inhibitory concentration; SOC, standard of care.

### Patient monitoring and follow-up

Biochemical analyses in serum (sedimentation rate, C reactive protein, haemoglobin, white blood cell count, platelet count, creatinine, alanine/aspartate aminotransferases) and synovial fluid, when appropriate in diagnostic work-up (white blood cell/neutrophil fraction, glucose and cultures), are performed at inclusion and 2, 4, 8 and 52 weeks after initial surgery. At these time points, patients will be examined and assessed by an expert orthopaedic surgeon and infectious diseases consultant. PROMs are obtained through Oxford Hip and Knee Scores<sup>45</sup> and EQ-5D-5L forms<sup>46</sup> at 1, 8 and 52 weeks after DAIR surgery. Study-specific case report forms are filled out at each visit and adverse events are registered and reported according to guidelines of the Swedish medical products agency. Participants are contacted by a study nurse prior to these visits, which are all conducted at one outpatient clinic.

### Timeline

For an overview of the study, see [figure 3](#). Patient inclusion, microbial analysis and patient data collection will be carried out between June 2021 and December 2022. The study ends when the last subject has completed the continuous 12-month follow-up, approximately in December 2023. The study may be prematurely terminated if the comparator treatment is associated to many serious events.

### Sample size calculation

We consider the diagnostic tool clinically useful if it guides treatment differently than standard scheme in more than 25% of the decisions, according to the MBEC cut-off for replacement. Change of treatment decision other than first-line treatment is considered as an event. Sample size calculation is based on two independent study groups comparing proportions of events. Based on our previous work,<sup>41</sup> we anticipate that MIC alone will guide to other than first-line treatment in 40% of the staphylococci PJI. We anticipate that the MIC and MBEC in combination will guide to other than first-line treatment in at least 75%

of cases. With 1:1 randomisation allocation, power 80% and alpha set to 5%, the study requires 60 patients, 30 in each group. We will include 64 patients to account for dropouts. Analyses will be undertaken according to intention to treat.

### Primary outcome measurements after 6 weeks of oral antimicrobial treatment

Proportions of antimicrobial regimens other than first-line treatment that will be administered after randomisation to either decision by MIC or MIC and MBEC testing combined.

### Secondary outcome measures at 12 months follow-up

(1) Repeat procedure, relapse or reinfection; (2) Oxford Hip Score/Oxford Knee Score; (3) EQ-5D: generic health status patient-reported outcome measure; (4) time to revision; (5) inpatient care: resource consumption measure (days); (6) outpatient visits: resource consumption measure, number of visits, type of visits; (7) discharge destination: resource consumption measure; (8) health care costs: compound measure using data from outcomes 6–8 (currency €); (9) development of additional antimicrobial resistance of the relapse causative strain; (10) correlation between the virulence properties of the causative bacteria and patient outcome (infection resolution vs recurrent infection).

### Patient and public involvement

Improving the success of implant preserving treatments in PJI is largely dependent on antibiotic efficacies. However, commonly used antibiotics have the potential for considerable side effects and drug interactions. Available regimens should therefore be optimised regarding exposure. Minimising unnecessarily prolonged or repeated treatments is of high importance for patients. Patients have not directly been involved in the study design. Intervention and follow-up are designed to follow normal clinical practices at our unit, in order not to impose unnecessary burden on study patients. Patients are made aware that the

study results will be published and may influence future practices, and that they can contact study managers for further information.

### Data analysis plan

Descriptive statistics will be presented as mean±SD, SE of the mean, median and IQR or mode and range. Proportion of treatments other than first-line treatment will be analysed by  $\chi^2$  test. The secondary outcome measures relapse free time and drug tolerability time for the two study arms and hazard ratios will be calculated with the Cox proportional hazard model.  $X^2$  test will be used for the analysis of 12-month resolution of infection in biofilm<sup>non/weak</sup> or biofilm<sup>strong</sup>, and MBEC<sup>low</sup> or MBEC<sup>high</sup>. MBEC/MIC ratios may not be considered normally distributed,<sup>41</sup> making the Mann-Whitney U test suitable in the analysis of relationships between MBEC/MIC ratios and clinical outcome, as well as in absolute value comparison of MBEC and MIC for each antimicrobial agent. Univariate logistic regression will be conducted to analyse biofilm production and clinical outcome. Statistical assistance will be obtained from the bioinformatics core facility at the University of Gothenburg.

### ETHICS AND DISSEMINATION

The study has received approval from the Swedish Ethical Review Authority, no 2020-01471, and the Swedish Medical Products Agency, EudraCT no 2020-003444-80. In case of significant protocol modifications, they will be reported within 7 days (from knowledge) to the Swedish Medical Products Agency, and amendment applications submitted. Informed (standard written Swedish form) consent will be obtained by the investigators or orthopaedic surgeons experienced in clinical research and thoroughly familiarised with the study. The sponsor will submit an annual safety report to the Swedish Medical Products Agency and will inform of the study's completion within 90 days. Within 1 year after study completion, the study results will be reported in the EudraCT database. Processed and interpreted outcome measures will be published in peer reviewed journals dedicated to clinical or translational research.

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involved in study design, protocol implementation at the orthopaedic infection unit, clinical data collection, analysis and interpretation and manuscript revision. MT, University of Gothenburg, Sahlgrenska Academy, department of biomaterials; principal investigator for the laboratory experimentation, involved in study design, microbiological method development, validation and implementation, microbiological data collection, analysis and interpretation and manuscript revision. JANT, Sahlgrenska University Hospital/Östra, department of infectious diseases, involved in study design, implementation of clinical protocol and treatment algorithm, clinical outcome data collection, analysis and interpretation and writing of manuscript. KSM, department of orthopaedics, Sahlgrenska University Hospital, involved in study patient inclusion, clinical data collection and manuscript revision. KR, department of orthopaedics, Sahlgrenska University Hospital, involved in study patient inclusion, clinical data collection and manuscript revision. SS, department of clinical microbiology, Sahlgrenska University Hospital, involved in clinical laboratory protocol design and implementation and manuscript revision. EL, department of clinical microbiology, Sahlgrenska University Hospital, involved in implementation of clinical laboratory protocol, validation of minimum inhibitory concentration assay, microbiological data collection and manuscript revision.

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