Diplomarbeit

Comparative analyses with drug-loaded cements for the usage in revision arthroplasty

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Julian Christopher Kunzi

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unter der Anleitung von

Prof. Dr. rer. nat. Klaus-Dieter Kühn

Univ.-Prof. Dr. med. univ. Andreas Leithner

Graz, am 26.07.2019

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Vorwort

Diese Diplomarbeit wurde im Rahmen einer Kooperation zwischen der Medizinischen Universität Graz (Universitätsklinik für Orthopädie und Traumatologie), dem Klinikum Charité – Universitätsmedizin Berlin (Abteilung für infektiöse Chirurgie) und der Firma Heraeus Medical GmbH in Wehrheim erstellt. Nachdem in dieser Arbeit Produkte von Heraeus Medical GmbH mit Konkurrenzprodukten verglichen werden, möchte ich hiermit versichern, dass diese Diplomarbeit und alle damit verbundenen Experimente und Berechnungen neutral und nach bestem Wissen und Gewissen erarbeitet wurden.

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Zusammenfassung

Einleitung

Die lokale Antibiotikatherapie bei periprothetischen Gelenksinfektionen zusammen mit einem ein- oder zweizeitigen Prothesenwechsel hat sich als essenziell für eine erfolgreiche Keimeliminierung erwiesen [Neut et al. 2007]. Studien und Case Reports zeigten allerdings, dass auch eine Rekolonisation und Biofilmbildung beim Einsatz von Gentamycin-haltigen Polymethylmethacrylaten (PMMA) möglich ist [Schmolders et al. 2014; Thornes et al. 2002]. Diese Untersuchung zeigt den in vitro getesteten Effekt von verschiedenen, in der Revisionschirurgie verwendeten, antibiotikabeladenen Zementen auf vier verschiedenen Keimen mittels Hemmhoftests.

Material/Methode

Elf handelsübliche, antibiotikabeladene (0,5-2,5 g Antibiotika in 40 g Pulver) Zemente wurden in fünf Testgruppen eingeteilt, in Formkörper gepresst und unter sterilen Bedingungen in 20 ml Phosphatpuffer inkubiert. Nach 1 h, 24 h, 7 d, 14 d, 21 d, 28 d und 42 d wurden die Formkörper entnommen und in frische Pufferlösungen überführt. Müller-Hinton-Agarplatten wurden mit MRSA, E. coli, *Pseudomonas aeruginosa* oder *Cutibacterium acnes* bestrichen und mittig mit einem 6 mm großen Loch versehen, in das 30 µl eines Eluats pipettiert wurden. Nach mindestens 24 Stunden Bebrütung bei 37 Grad Celsius wurden die Hemmhöfe mit einem Lineal vermessen. Jeder Zement wurde dreimal mit einem Keim getestet. Alle Hemmhofgrößen wurden in Tabellen, Grafiken und Fotos festgehalten. Aufgrund des geringen Umfangs wurde ausschließlich eine deskriptive Statistik durchgeführt.

Ergebnisse

Gruppe A mit Aminoglykosiden: Kein Zement erreichte einen Effekt gegen MRSA. Gegen E. coli zeigte Palacos® R+G stets den besten Effekt und wirkte als einziger noch nach 42 Tagen. Hi-Fatigue®, Simplex® HVG und BonOs R Genta zeigten bis zum 28. Tag einen antibakteriellen Effekt, während Simplex® T über maximal 21 Tage und CMW™ 1 G bis zu sieben Tage wirksam war (Abbildung 1). Auch die Gruppe-B-Zemente mit Gentamycin und Clindamycin zeigten keine Wirkung gegen MRSA. Refobacin® Revision und Copal® G+C hatten sehr ähnliche Hemmhöfe gegen *Cutibacterium acnes* mit einem Maximum

nach sieben Tagen mit 63,7 mm ± 1,5, beziehungsweise 63,3 mm ± 2,9. Simplex® E+C (Gruppe C) war unwirksam gegen *Pseudomonas aeruginosa* und MRSA. In Gruppe D mit Gentamycin und Vancomycin waren signifikante Unterschiede zu beobachten. Gegen E. coli wirkte Vancogenx® nur bis zum siebten Tag, gegen MRSA maximal bis zum 21. Tag. Copal® G+V wirkte stets über 42 Tage (Abbildung 2) bei beiden ausgewählten Keimisolaten. Gruppe E mit Copal® G+C erreichte gegen E. coli sehr ähnliche Ergebnisse wie die Tests gegen *Cutibacterium acnes* aus Gruppe B.

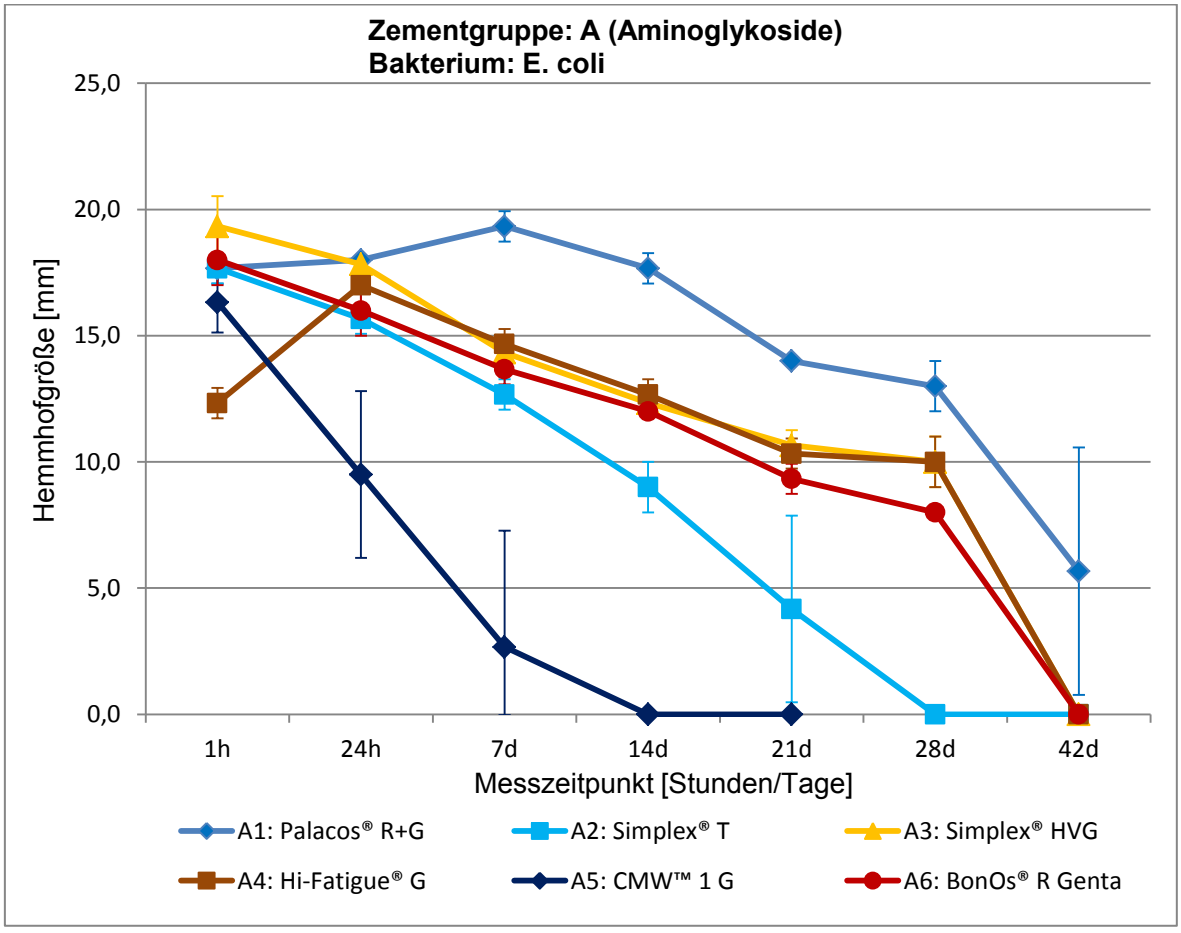


Abbildung 1: Gemittelte Hemmhofgrößen mit Standardabweichung der Gruppe-A-Zemente gemessen mittels Agardiffusionstests gegen E. coli.

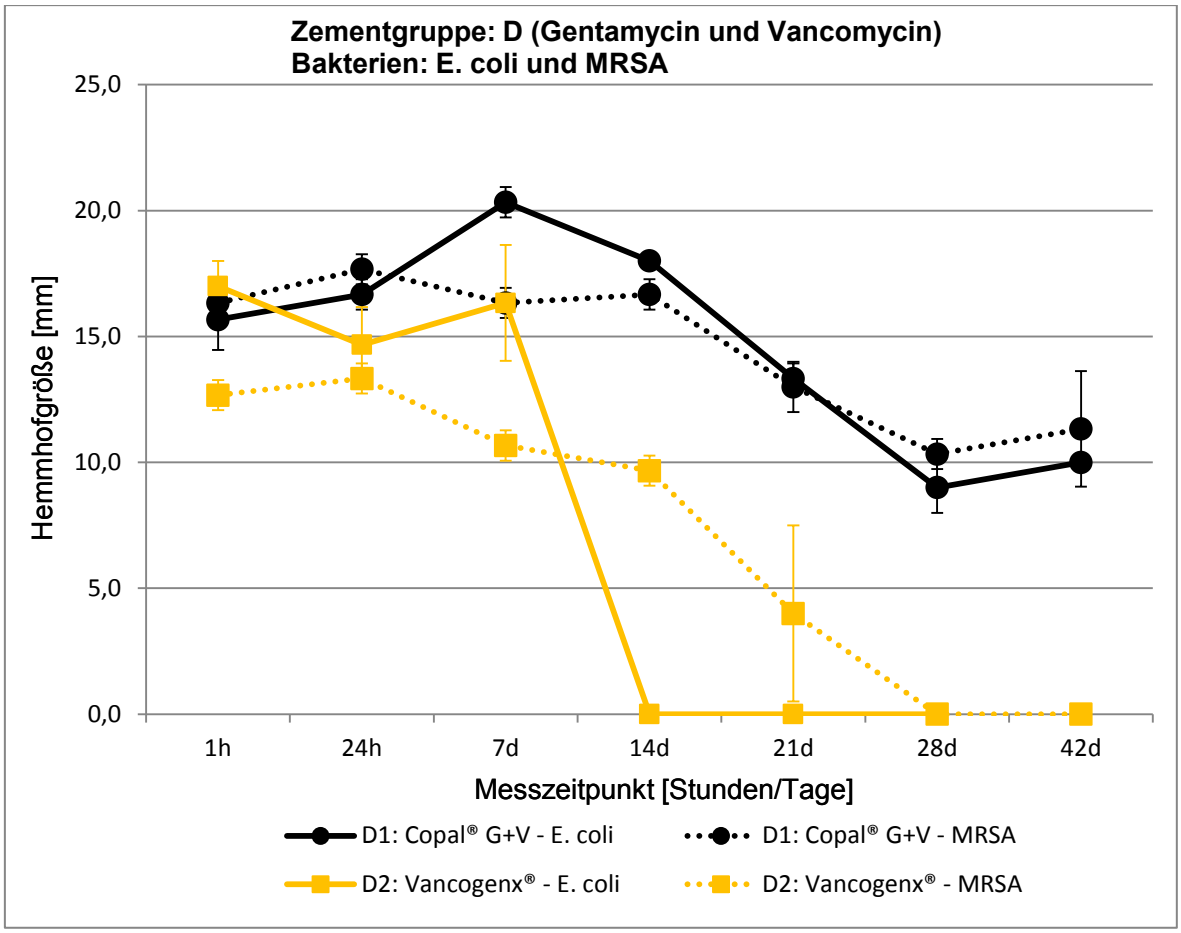


Abbildung 2: Gemittelte Hemmhofgrößen mit Standardabweichung der PMMA-Zemente aus Gruppe D gemessen mittels Agardiffusionstests gegen E. coli und MRSA.

Diskussion

Die Ergebnisse zeigen deutliche Unterschiede bei den getesteten PMMA-Zementen. Um Rekolonisation und Resistenzbildung und damit verlängerte Klinikaufenthalte sowie zusätzliche Operationen zu vermeiden, sollte auf die Wahl des PMMA Knochenzements geachtet werden, weil diese offenbar die Antibiotika sehr unterschiedlich freisetzen und dadurch die Wirksamkeit gegen Keimisolate ebenfalls sehr unterschiedlich sein kann. Die Studie zeigt zudem, dass die Antibiotikakonzentration im Pulver keine Aussage über den antibakteriellen Effekt des Zements zulässt. Das Freisetzungsverhalten und damit die mikrobiologische Wirksamkeit der Zemente ist für Zumischempfehlungen der PRO-IMPLANT-Foundation bei Revisionen von Bedeutung [Renz und Trampuz 2018].

Abstract

Introduction

The therapy with local antibiotics by periprosthetic joint infections in combination with a one-stage or two-stage exchange of the prosthesis is essential for a successful eradication of the bacteria [Neut et al. 2007]. However, studies and case reports show that a recolonization and formation of biofilms is also possible by the use of gentamicin-containing polymethylmethacrylates (PMMA) [Schmolders et al. 2014; Thornes et al. 2002]. This investigation tested the in vitro effect of different drug-loaded bone cements which were used in revision surgery against different microbes with agar diffusion tests.

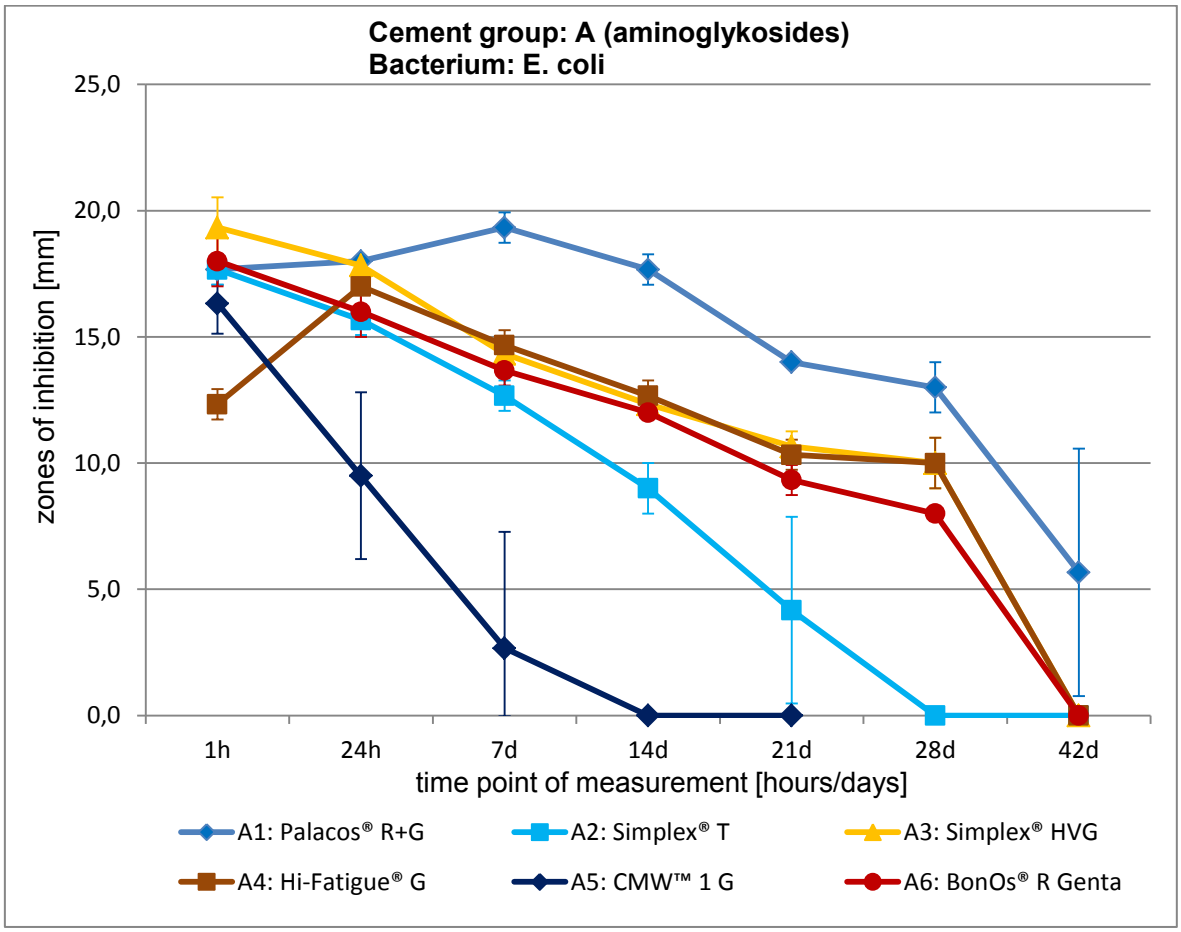
Material/Methods

Eleven drug-loaded (0.5-2.5 g antibiotics in 40 g powder) cements were categorized in five groups, pressed in moulds and incubated in 20 ml phosphate buffered saline solution under sterile conditions. The cement moulds were put out, dried and inserted in a new solution after 1 h, 24 h, 7 d, 14 d, 21 d, 28 d and 42 d. Mueller-Hinton agar plates were spread with MRSA, E. coli, *Pseudomonas aeruginosa* or *Cutibacterium acnes* and a hole with a diameter of six millimeters was punched in the centre of each plate and filled with 30 µl of one of the elution fluids. After at least 24 hours incubation by 37 degrees Celsius, the zones of inhibition were measured with a ruler. Each bone cement was tested three times against every bacterium. The inhibiting areolas were documented in tables, graphs and pictures. Solely descriptive statistics were calculated because of the small number of test repetition.

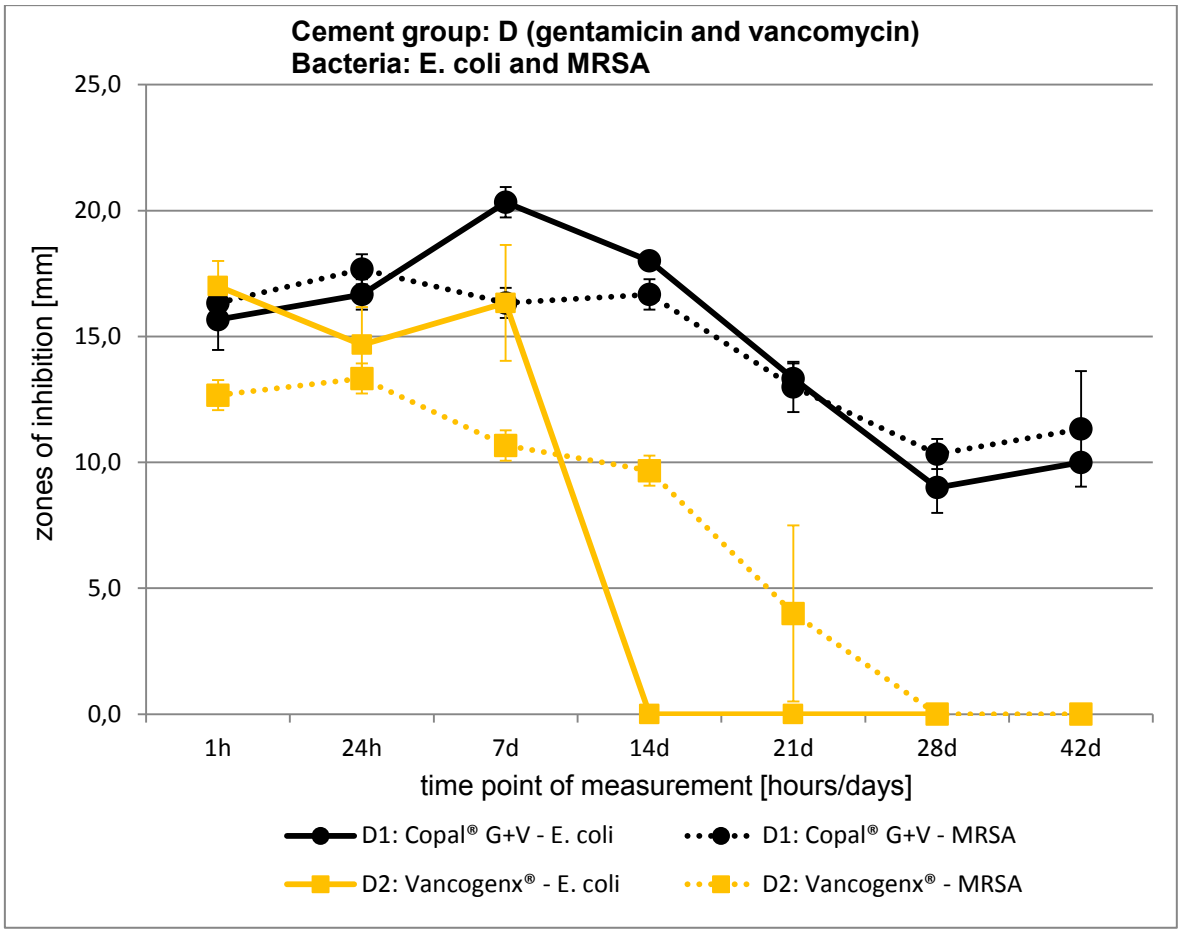
Results

Group A contained PMMAs with aminoglycosides. None of the cements reached any effect against MRSA. Palacos® R+G showed always peak values and was the only one that had an antibacterial efficacy of 42 days. Simplex® HVG, Hi-Fatigue® G and BonOs® R Genta reached good zones of inhibition until the 28th day while Simplex® T showed inhibiting areolas for 14-21 days and CMW 1 G only for 24 hours to 7 days (graph 3). Cements of group B with gentamicin and clindamycin also showed no effect against MRSA. The inhibition zones of Refobacin® Revision and Copal® G+C with *Cutibacterium acnes* were very similar and reached a peak value on the seventh day with 63.7 mm ± 1.5 and 63.3 mm ± 2.9, respectively. Simplex® E+C (group C) reached no effect against

Pseudomonas aeruginosa and MRSA. There were significant differences between the PMMAs in group D which contained gentamicin and vancomycin. Vancogenx® showed an effect against E. coli until day seven and against MRSA until the 21st day at most. Copal® G+V had a good antibacterial efficacy over the whole tested time period with both tested bacterial strains (graph 4). Group E with Copal® G+C showed similar results like in the tests against *Cutibacterium acnes* of group B.



Graph 3: Mean antibacterial efficacy and standard variation of the antibiotic in all cements of group A which were tested with *Escherichia coli*.



Graph 4: Mean antibacterial efficacy and standard variation of the antibiotic in all cements of group D which were tested with *Escherichia coli* and MRSA.

Discussion

The results showed clear differences between the tested PMMA cements. The choice of the bone cement should be made in a considerate way, because the antibiotic elution of the various brands obviously differs very much which can lead to different antibacterial efficacy. Furthermore, the study shows that the concentration of antibiotic in the cement powder does not allow any interpretation to the antibacterial efficacy. The quantity of drug release and thereby the microbiological efficacy is important for recommendations of the PRO-IMPLANT-Foundation for admixture in revision surgery [Renz and Trampuz 2018].

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List of abbreviations

ATCC = American Type Culture Collection

C. acnes = *Cutibacterium acnes*

cm = centimeter(s)

cm^3 = cubic centimeter(s)

Con. = continuous

d = day(s)

E. coli = *Escherichia coli*

ed. = editor

eds. = editors

e.g. = exempli gratia

EPS = extracellular polymeric substances

EUCAST = European Committee on Antimicrobial Susceptibility Testing

g = gram(s)

h = hour(s)

ISO = International Organization for Standardization

l = liter(s)

mg = milligram(s)

MIC = minimum inhibitory concentration

ml = milliliter(s)

MRSA = methicillin-resistant *Staphylococcus aureus*

MSSA = methicillin-sensitive *Staphylococcus aureus*

MSIS = MusculoSkeletal Infection Society

µg = microgram(s)

µl = microliter(s)

p. = page(s)

PJI = periprosthetic joint infection

PMMA = polymethylmethacrylate

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1 Introduction

Orthopaedics is a diversified speciality of human medicine. While illnesses of children earlier shaped this medical field, which is where the name is derived from, adult patients are becoming more frequent due to demographic changes in the last centuries. The main suffering of these elder patients is a degenerative illness of hip and knee joints. That is why the focus of this thesis lies on the use of antibiotic-loaded cements in hip and knee replacement.

1.1 Arthrosis and endoprosthetics

Arthrosis is a degenerative and irreversible illness of joints which is primarily not infectious and results in joint destruction. Epidemiologic statements cannot be made easily, because of different diagnostic criteria. While Scharf et al. [2008] described the prevalence of radiologically diagnosed arthrosis in 10-20% of the European population, Ficklscherer [2017: p.82] described the prevalence of arthrosis in Germany in three different groups: 15% of the 35-44-year-olds, 40% of the 55-64-year-olds and 60% of the 75-84-year-olds. Women are affected more often than men. The cause of arthrosis is mainly a disproportion between pressure and capacity. Hip and knee joints are mostly affected. The treatment complies with the dimension of the destruction of the cartilage. Besides weight reduction, physiotherapy, medication and osteotomy, the treatment of especially elder patients with higher cartilage damage is a surgical replacement of the joint. Although the insertion of a total prosthesis is the ultima ratio of the treatment, it is a common standard practice nowadays. Besides traumatic joint fractures, arthrosis is therefore the main indication for a surgical treatment with replacement of the joint.

While authors of German orthopaedic books agree on the numbers of primary hip replacements about 200.000 to 210.000 per year in Germany, there are different statements to the numbers of primary knee joint replacements from 60.000 to 170.000 per year in Germany [Ficklscherer 2017: p. 86; Breusch et al. 2017: p. 428/494; Grifka/Krämer 2013: p. 76]. An increase of the numbers is generally expected for the next years because of demographic changes.

Like every invasive treatment, the total joint replacement of knee or hip can cause complications. In detail these are high loss of blood, nerve lesion, vessel lesion, embolism, thrombosis, periprosthetic joint infection, periprosthetic fracture, tendency to luxation,

different length of the legs, periarticular calcification and aseptic loosening of the prosthesis [Müller 2014: p. 395]

1.2 Periprosthetic joint infections (PJI)

With 0.3 to 1.8 percent in knee replacements and 0.6 to 1.7 percent in hip arthroplasties, PJI are a rare complication, but according to Del Pazo and Patel [2009] they are the most serious [Choong et al. 2007; Jämsen et al. 2009; Peersman et al. 2001; Phillips et al. 2006; Pulido et al. 2008]. An early diagnosis and the safe treatment of the infection are important for the patient's outcome.

1.2.1 Pathogenesis

The literature distinguishes between different pathogenic origins of an infection. The first and most common option is an infection where the bacteria invade into the body joint while arthroplasty surgery. Especially skin bacteria enter the joint in this way. Second is a delayed infection where the bacteria enter the host at a different place and colonise the joint via haematogenetic spread. Furthermore, Del Pozo and Patel [2009] describe a third origin of an infection through compromised local tissue [Prokuski 2008; Renz and Trampuz 2017; Stavrakis et al. 2013].

When the bacteria settle down on the surface of the prosthesis, they start to multiply and build up a biofilm. The endoprosthesis supports the growing of the germs, because it is a kind of retreat for the germs and the immune defence is not able to identify the germs. Biofilms consist of five to thirty-five percent of bacteria cells. The other parts are summarized as extracellular polymeric substances (EPS) which are mostly produced by the bacterial cells themselves. This biofilm formation optimizes the whole life cycle of bacteria. Especially the improvement of the adhesion to the surface, the intercellular binding, the three-dimensional architecture and especially the resistance against antibiotics and the human immune system are beneficial factors for PJI [Del Pozo and Patel 2007/2009; Flemming and Wingender 2010; Trampuz and Widmer 2006].

1.2.2 Categorization

Apart from the differentiation regarding the origin of the bacteria, PJIs were classified according to four other criteria: pathogens, status of the soft tissue, diagnostic probability and time period.

The determination of the causing germs is essential for a sufficient therapy and therefore important to categorize. The main representatives of pathogens are staphylococcal species (58-70%), especially *Staphylococcus epidermidis* (15-43%) and *Staphylococcus aureus* (12-42%) [Mooney et al. 2018; Peersman et al. 2001; Pulido et al. 2008; Stavrakis et al. 2013; Triantafyllopoulos 2018]. Germs which are resistant to biofilm-active antimicrobials cause infections which are difficult to treat. Renz and Trampuz [2017] summarize rifampicin-resistant staphylococci, ciprofloxacin-resistant gram-negative bacteria and fungi to this group, while Zimmerli et al. [2004] describe multi-drug resistant bacteria, enterococcus species and fungi as difficult to treat.

The categorization according to diagnosis can be made with a score which was designed by Zimmerli and Ochsner [2003] to objectify the probability of the existence of an infection. The score evaluates clinical signs, radiologic diagnostic and laboratory values.

The beginning of the clinical manifestation of the infection divides PJIs in acute and chronic. There are two different opinions in literature. Some authors describe acute infections with first symptoms within four weeks after joint replacement or other causative intervention and after that, the infection is chronic [Renz and Trampuz 2017; Stavrakis et al. 2013]. The others differ acute from chronic infection with the time limit of three months, or rather ninety days [Del Pozo and Patel 2009; Parvizi et al. 2014]. In addition, Betsch et al. [2008], Schafroth et al. [2003], Wouthuyzen-Bakkar et al. [2018] and Zimmerli et al. [2004] separate the chronic infections in delayed (three to twenty-four months) and late onset (more than twenty-four months).

This distinction is assumed to be a result of the virulence of the bacteria and therefore important for the specific treatment. High-virulent bacteria like *Staphylococcus aureus*, E.coli or *Pseudomonas aeruginosa* cause acute infections, while low-virulent bacteria like *Staphylococcus epidermidis* and *Cutibacterium acnes* make chronic infections which are very difficult to treat, because their biofilms are mature in contrast to acute infections [Renz and Trampuz 2017; Stavrakis et al. 2013].

1.2.3 Risk factors

Risk factors for periprosthetic joint infection can be divided in three groups: patient-related risk factors, intraoperative risk factors and postoperative risk factors. The first group consists of male gender, tobacco or alcohol abuse, obesity, poor nutrition, hypopotassaemia, pulmonary hypertension, coronary artery disease, diverticulosis, renal failure, hypothyroidism, arthritic diseases, neoplasm, immunosuppression, diabetes mellitus, lupus erythematosus, haemophilia and avascular necrosis or previous arthroplasty at the same joint. Intraoperative risk factors are prior open surgical procedure, prolonged surgery time with over 1.5 hours, simultaneous bilateral joint replacement and allogenic blood transfusion. Anaesthetic complications, prolonged hospital stay, wound healing complications, atrial fibrillation, myocardial infarction, infection of the urinary system and infection with *Staphylococcus aureus* were summarized as postoperative risk factors [Del Pozo and Patel 2009; Jämsen et al. 2009; Peersman et al. 2001; Triantafyllopoulos 2018; Wang et al. 2019].

1.2.4 Prevention

There are some basic prevention strategies which are recommended by many authors. The patient should be loaded with an antibiotic preoperatively and twenty-four hours postoperatively, e.g. cefazolin or cefuroxime [Prokuski 2008]. A combined use of antibiotics, intravenous and local with drug-loaded cements is requested. The operation theatre should have a vertical laminar air flow and body exhaust suits. The overall infection rate reduces thereby to 0.43 percent [Jämsen et al. 2009; Peersman et al. 2001; Pulido et al. 2008].

1.2.5 Diagnose

There is no standardized definition of PJI in literature, but the experts' opinions can be summarized in two different definitions. The first definition was published in many papers over the last three decades. Therefore, a PJI is present when one of the following criteria has been met:

- Acute inflammation in histopathological examination of periprosthetic tissue
- Sinus tract communicating with the joint
- Purulence around the prosthesis

- Bacterial growth in synovial fluid, sonication fluid or at least two tissue samples

[Berbari et al. 1998; Betsch et al. 2008; Piper et al. 2009; Renz and Trampuz 2017; Trampuz et al. 2007].

The definition of the MusculoSkeletal Infection Society (MSIS) from 2014 (table 1) represents the opinion of four hundred experts from fifty-two countries. These criteria were made for knee and hip joint and are limited for traumatic joint replacements, oncological surgery and replacements of other joints [Parvizi 2014 and 2017; Triantafyllopoulos 2018].

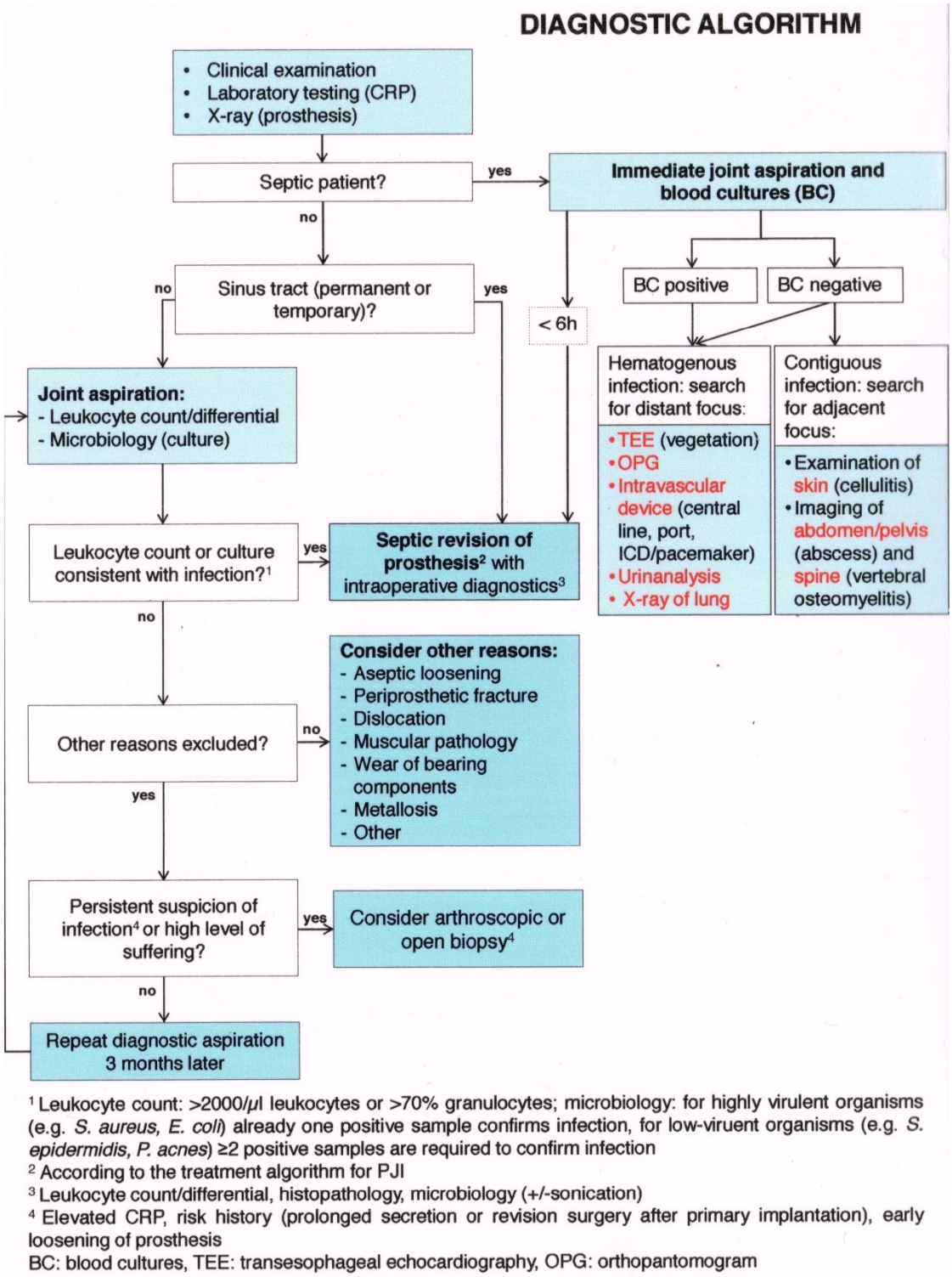
PJI persists when one major criterion or three minor criteria are fulfilled.		
	Acute PJI	Chronic PJI
Major Criteria		
Two positive periprosthetic cultures with phenotypically identical organisms		
A sinus tract communicating with the joint		
Minor Criteria		
Elevated serum C-reactive protein (CRP) and erythrocyte sedimentation rate	100 mg/l No threshold	10 mg/l 30 mm/h
Elevated synovial fluid white blood cell count or change on leukocyte esterase test strip	10,000 cells/µl + or ++	3,000 cells/µl + or ++
Elevated synovial fluid polymorphonuclear neutrophil percentage	90 %	80%
A single positive culture		
Positive histological analysis of periprosthetic tissue	>5 neutrophils per high power field in 5 high power fields (x400)	

Table 1: Definition of PJI of the MusculoSkeletal Infection Society. Modified from Parvizi et al. [2014].

Esposito et al. [2009] and Renz and Trampuz [2017] created different algorithms for single diagnostic steps. When a patient has pain in a prosthetic joint, Esposito et al. [2009] recommend X-ray diagnosis and/or physical examination with focus on signs of infection (rubor, calor, dolor, tumor, functio laesa) and presence of a sinus tract. If these tests suggest a PJI, the next steps are arthrocentesis and laboratory tests of blood and synovial fluid, followed by a triphasic scintigraphy and scintigraphy with labelled leucocytes. Only

if all results suggest a PJI, the infection is treated. Otherwise, a more general diagnosis or revaluation one to two months later is necessary [Esposito et al. 2009].

The second algorithm is shown in graph 5.



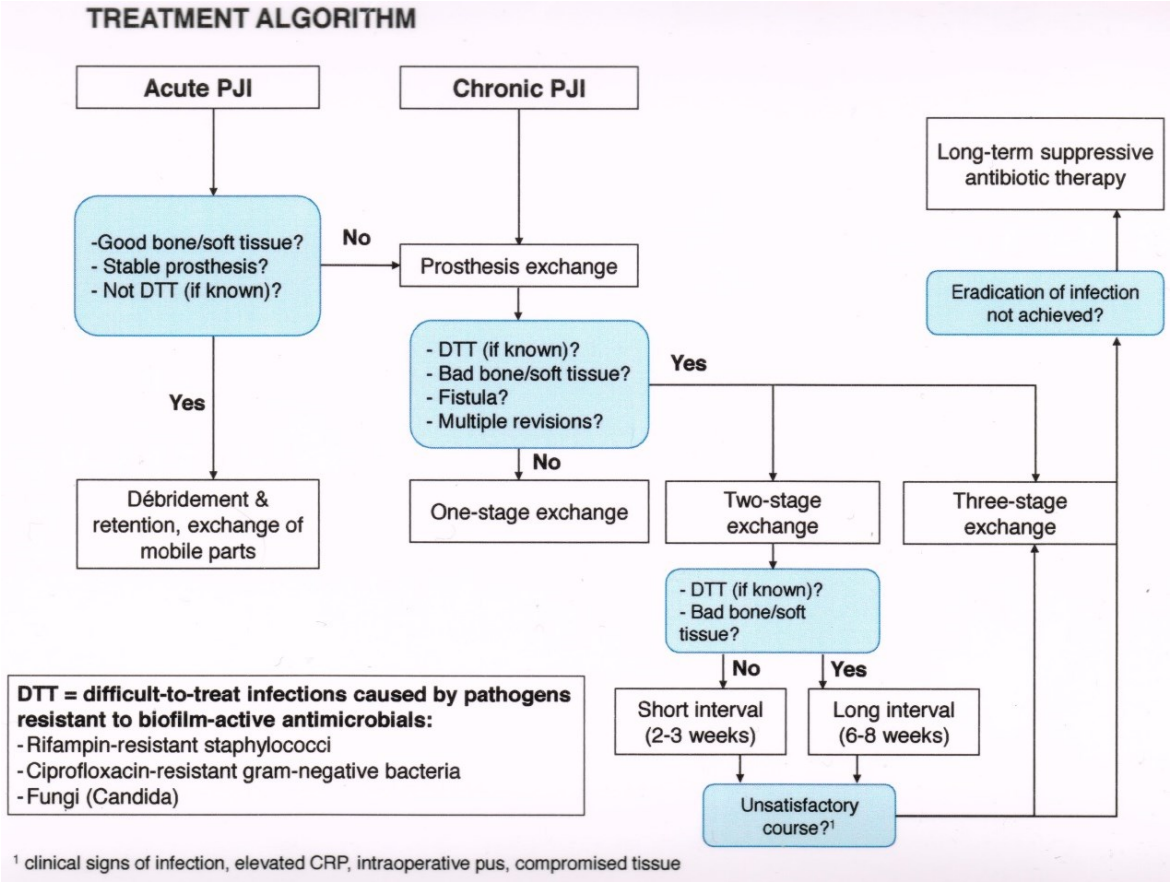
[1] Leukocyte count: >2000/µl leukocytes or >70% granulocytes; microbiology: for highly virulent organisms (e.g. *S. aureus, E. coli*) already one positive sample confirms infection, for low-viruent organisms (e.g. *S. epidermidis, P. acnes*) ≥2 positive samples are required to confirm infection
[2] According to the treatment algorithm for PJI
[3] Leukocyte count/differential, histopathology, microbiology (+/-sonication)
[4] Elevated CRP, risk history (prolonged secretion or revision surgery after primary implantation), early loosening of prosthesis
BC: blood cultures, TEE: transesophageal echocardiography, OPG: orthopantomogram

Graph 5: Diagnostic algorithm for PJI according to pocket guide to diagnosis & treatment of periprosthetic joint infection (PJI), version nine, April 1st, 2018 [Renz and Trampuz 2018].

The treatment of periprosthetic joint infections is difficult and much discussed. There are a few possibilities: debridement with exchange of the mobile parts of the prosthesis, one-stage revision, different procedures of two-stage revision, three-stage revision, long-term application of antibiotics, removal of prosthesis without replacement (arthrodesis) and amputation [Del Pozo and Patel 2009; Renz and Trampuz 2017].
Del Pozo and Patel [2009] published the following recommendation:

- When the patient cannot be operated, he receives a long-term therapy with antibiotics in a palliative approach.
- When there is no expectation that a revision achieves a little functional improvement or multiple attempts to cure the infection had failed, a removal of the implant without replacement in combination with an antimicrobial therapy is recommended. If the infection is uncontrollable, an amputation could be necessary.
- When the infection occurs within three months after joint replacement or the infection has a haematogenous origin, the symptoms present less than three weeks, there is no abscess or sinus tract, the implant is stable and the bacteria are not difficult to treat, the patient receives a debridement with exchange of the mobile parts and an antimicrobial treatment.
- Otherwise a one- or two-stage revision is necessary.

Renz and Trampuz [2018] have another recommendation. They differ acute from chronic PJI with the time limit of four weeks (graph 6).



Graph 6: Treatment algorithm for PJI according to pocket guide to diagnosis & treatment of periprosthetic joint infection (PJI), version nine, April 1st, 2018 [Renz and Trampuz 2018].

1.2.5.1 Debridement and exchange of mobile parts

The surgical debridement of an infectious joint has to be performed very thorough. It includes the excision of sinuses, synovectomy and the removal of pus, infected haematoma, foreign materials, tissue films on the surface of the prosthesis and soft tissue which is damaged through infection, inflammation or necrosis. The mobile parts are exchanged and the joint space flushed out with lots of sterile isotonic saline solution or disinfecting solution. The stable implant remains in the patient. An administration of antibiotics is necessary afterwards. Renz and Trampuz [2018] recommend intravenously administered antibiotics without antibiofilm activity for two weeks and then oral antibiotics with antibiofilm activity for ten weeks. The debridement can also be repeated [Choong et al. 2007; Von Foerster and Frommelt 2009].

1.2.5.2 One-stage revision

This surgery is only recommended, when the infection causing bacteria has been identified. Otherwise, a two-stage revision is considered safer to eradicate PJI [Gaston 2007; Von Foerster and Frommelt 2009].

One-stage revision surgery includes the removal of the prosthesis, debridement and the insertion of a new prosthesis. The use of cemented prosthesis with antibiotic-loaded polymethylmethacrylate (PMMA) and the change of gowns and surgical equipment between step two and three is recommended to minimize the risk of re-infection [Chew et al. 2015; Meani and Trezza 2009].

Renz and Trampuz [2018] recommend the same antimicrobial treatment as after debridement and retention of the prosthesis (1.2.6.1), while Esposito et al. [2009] and Zimmerli et al. [2008] recommend the start of antibiotic treatment two to three weeks before one-stage revision, if the pathogen is identified and the patient has no severe systemic infection.

1.2.5.3 Two-stage revision

The main concept of a two-stage revision is the explantation of the prosthesis and debridement of the joint space in a first operation followed by de novo debridement and implantation of a new prosthesis in a second operation after some weeks of antibiotic therapy. The time interval between the surgeries can be bridged in four different ways: antimicrobial treatment combined with drug-loaded cement block spacer, antibiotic-loaded articulating spacer, antibiotic-impregnated cancellous bone allograft or without spacer.

The use of an articulating drug-loaded spacer is most commonly used and recommended, because there are lots of advantages. It keeps the joint space clear from fibrous tissue, the patient's leg has the normal length, can be moved and bear partial weight which prevents stiffness, bone atrophy and ligament contracture. This benefits wound healing and simplifies reimplantation. Furthermore, the local concentration of antibiotics is much higher than with systemic antibiosis only. The duration of hospitalization and the operative time are shorter than in revisions with antibiotic-loaded PMMA beads and loss of blood as well as the number of needed transfusions are lesser. The foreign material in the patient is a disadvantage, because it can benefit the bacterial growth, when the antibiotic is consumed. Furthermore, the spacer can be painful and

dislocate easily [Eberhardt 2009; Hofmann et al. 2005; Hsieh et al. 2004; Romanò et al. 2012; Verdonk et al. 2009; Zimmerli et al. 2004].

The two-stage exchange can be classified in short and long interval between both operations. The choice of the interim is made after infection causing germs and status of bone and soft tissue have been determined (graph 6). The time period for short and long time interval operations is described differently: Renz and Trampuz [2018] recommend two to three weeks for short interval and six to eight weeks for long interval, Del Pozo and Patel [2009] recommend six and twelve weeks, respectively and Gaston [2007] andMalizos et al. [2007] recommend only one interval with eight and eleven weeks, respectively. Vielgut et al. [2015] recommend a time period of four to eleven weeks to minimize the risk of reinfection. Hsieh et al. [2004] perform the second stage only if the patient is well enough for surgery, the wound has healed and erythrocyte sedimentation rate and serum C-reactive protein level has normalised.

1.2.5.4 Three-stage revision

After an explantation surgery with debridement and implantation of a drug-loaded spacer, the patient receives antibiotics without antibiofilm activity intravenously for three weeks. Then, a second surgery is performed with exchange of the spacer and debridement, followed by the same antimicrobial treatment again. Afterwards the patient is operated with debridement and implantation of a new prosthesis. Finally, a third antibiotic therapy is recommended with intravenously administered antibiotics without antibiofilm activity for one week and afterwards oral antibiotics with antibiofilm activity for five weeks [Renz and Trampuz 2018].

Konstantinos et al. [2007] switch from two-stage to three-stage revision, if multiple histologic samples which are removed during the second surgery, show more than five polymorphonucleates in five high power fields in each taken sample. The recommendation for the period of time between the surgeries is two to three months.

1.2.5.5 Resection arthroplasty, arthrodesis and amputation

All three surgeries, mentioned above, are rarely indicated in comparison to the other described procedures. While resection arthroplasty and arthrodesis can remain only for a certain time, an amputation is final.

Resection arthroplasty of the hip, also called Girdlestone pseudarthrosis, is a successful procedure to eradicate PJI. Castellanos et al. [1997] describe healing in 86 percent of patients with failed debridement, one-stage or two-stage revision. This surgery includes the removal of the prosthesis and all foreign material and the debridement of the joint space which is left empty. After surgery, the space fills up with scar tissue which allows painless motion. Apart from antimicrobial treatment, the aftercare is described differently. While Jahoda [2009] recommends skeletal traction and long-term immobilization, Castellanos et al. [1997] describe skin traction and contemporary rehabilitation. Walenkamp [2017] recommends only mobilization. After Girdlestone pseudarthrosis, every patient needs at least one crutch for walking. Moreover, walking is only possible, if they are capable. The operated leg is up to eight centimetres shorter and mostly forty to ninety degrees in external rotation [Castellanos et al. 1997; Hanssen and Spangehl 2004, Jahoda 2009; Walenkamp 2007].

While resection arthroplasty is the common choice for the hip joint, the knee joint is treated with arthrodesis surgery, when debridement or revisions have failed. There are four different possibilities to stiffen the knee joint: external fixator, Ilizarov fixator, plate fixation or intramedullary nail. A combination of external fixation (straight or circular) or resection arthroplasty of the knee joint with a plate fixation or intramedullary nail is possible in a two-stage procedure. Persisting infections and inadequate bone contact can cause failure of the arthrodesis. In that case, a re-arthrodesis or amputation is needed [Van Rensch et al. 2012].

An amputation can be necessary to treat a life-threatening infection, failed arthrodesis, vascular injury or massive loss of bone and soft tissue, while the latter can also be treated with tibia-hindfoot osteomusculocutaneous rotationplasty with calcaneopelvic arthrodesis, if the patient's remaining leg below the hip joint is in good condition [Hanssen and Spangehl 2004; Peterson et al. 1997].

1.2.5.6 Systemic antibiotics

Every treatment of PJI is combined with an antibiotic therapy. Even lifelong antimicrobial treatment can be necessary for patients who cannot be operated.

The choice of the right antibiotic and the length of administration are discussed extensively. Lots of authors designed different detailed tables with their recommendations (e.g. Gaston [2007], Esposito et al. [2009], Osmon et al. [2013], Renz and Trampuz [2017], Zimmerli and Ochsner [2003], Zimmerli et al. [2004]).

In general, it is recommended to choose the antibiosis after identification of microorganism and susceptibility check. Patient's allergies and intolerances have to be considered. The administered dose depends on weight as well as renal and hepatic function. If antibiotics with nephrotoxic or ototoxic side effects like vancomycin are given, the dose has to be monitored with serum concentration. The route of administration should first be intravenously and then switch to oral medication [Gaston 2007; Osmon et al. [2013]; Renz and Trampuz 2017].

1.3 Local antibiotics

The usage of a local antimicrobial therapy is highly recommended in patients with infections. Many authors also recommend using local antibiotics as prophylaxis to minimize the risk of a feared periprosthetic infection (e.g. Amerstorfer et al. [2017]; Colas et al. [2015]; Engesaeter et al. [2003]; Hendriks et al. [2004], Kühn et al. [2016], Martínez-Moreno et al. [2017], Parvizi et al. [2008], Sprowson et al. [2016]; Wang et al. [2013]).

There are lots of different carriers for antibiotics for a local treatment: bone grafts, bone substitute materials, collagen sponges, powders, drug-coated prosthesis, polymers, composite biomaterials and bone cements, whereby the last is the most researched and recommended drug delivery system. PMMA bone cements differ in many characteristics. Acrylic cements with an in vitro polymerization temperature of about 105 degrees Celsius and medium or high viscosity have the best test results [Spierings 2007]. Two different types are basically available by various producers: antibiotic-containing and plain acrylic cements. Both types can be manually mixed with additional antibiotics which should be used in powdered form and fulfil special physical and chemical characteristics. Commercial available drug-loaded products contain gentamicin, tobramycin, gentamicin and vancomycin, gentamicin and clindamycin or erythromycin and colistin. The characteristics of the PMMA and the added antibiotics determine the release performance. PMMAs can be prepared in ambient pressure or with help of vacuum mixing systems which can change the mechanical characteristics and the elution of antibiotics, too [Górecki and Babiak 2009; Kühn et al. 2017].

The local antibiotic therapy should always be matched with the infection causing microbes and their susceptibility. If a readily mixed cement containing a suitable antibiotic is available on the market, it should be preferred in comparison with self-mixed PMMA, because these cements comply with international industrial standards (ISO 5833) and are

safe for usage. Otherwise it is recommended for the surgeon to mix the needed antibiotic to the powder of the acrylic cement himself/herself. In the best case, the cement already contains an industrial mixed antibiotic to increase the drug release. If surgeons admix drugs manually, they become the manufacturer and bear the liability [Kühn et al. 2017; Malhotra et al. 2018].

Case reports and studies have shown that not every local antimicrobial treatment is successful [Kendall et al. 1996; Swieringa and Tulp 2005; Van Raaij et al. 2004; Schmolders et al. 2014; Thornes et al. 2002; Wu et al. 2009]. Bacterial colonisations of drug-loaded cement spacers are described and even nephrotoxic serum level are possible by incorrect and excessive usage. Comparative analyses with antibiotic-containing PMMA have shown that these products differ strongly [Kühn et al. 2016].

1.4 Aim of this study

This study investigated the microbiological in vitro efficacy of different antibiotics in commercially available polymethylmethacrylate (PMMA) bone cements for the usage in revision arthroplasty. The testing and evaluation of biomechanical qualities like strength, roughness of the surface, fatigue or porosity were not part of the study, because Klekamp et al. [1999] describe six percent decrease of the compressive strength by three grams of antibiotics in forty grams of cement powder. Lautenschlager et al. [1976] and Mohd Fuad et al. [2006] describe a loss of strength only if more than 4.5 grams of antibiotics are used in the powder. The cements which were tested in this study originally contain maximal 2.5 grams (0.5-2.5 g/40 g) of antibiotics. Because the antibiotics within the tested cements are not admixed manually but industrially, no biomechanical restrictions are expected.

Therefore, we focused on PMMA bone cements which are mainly recommended for revision arthroplasty and answer the question if there is a significant difference between the antibacterial efficacies of these antibiotic-loaded PMMAs.

2 Material and methods

Cement moulds were produced and tested in the laboratory of Heraeus Medical GmbH (Wehrheim, Germany) and the laboratory of university hospital Charité (Berlin, Germany).

2.1 Cement moulds

Eleven different drug-loaded cements which are currently available on the market were tested in this study. All products used for this investigation are shown in table 2. They were classified in five different groups due to their containing antibiotics.

	Product name:	**Producer:**	**Containing antibiotics in 40 g powder:**
Group A:	Palacos® R+G	Heraeus Medical GmbH (Germany)	0.5 g gentamicin
	Simplex® T	Stryker® (USA)	1.0 g tobramycin
	Simplex® HVG	Stryker® (USA)	0.5 g gentamicin
	Hi-Fatigue® G	Zimmer Biomet® (USA)	0.55 g gentamicin
	CMW™ 1 G	DePuy Synthes (USA)	1.0 g gentamicin
	BonOs® R Genta	aap Biomaterials GmbH & Co. KG (Germany) (now Osartis GmbH)	0.5 g gentamicin
Group B:	Copal® G+C	Heraeus Medical GmbH (Germany)	1.0 g gentamicin, 1.0 g clindamycin
	Refobacin® Revision	Zimmer Biomet® (USA)	1.0 g gentamicin, 1.0 g clindamycin
Group C:	Simplex® E+C	Stryker® (USA)	0.5 g erythromycin, 0.24 g colistin

Table 2: All tested products and their allocation.

Group D:	Copal® G+V	Heraeus Medical GmbH (Germany)	0.5 g gentamicin, 2.0 g vancomycin
	Vancogenx®	Tecres® (Italy)	1.0 g gentamicin, 1.0 g vancomycin
Group E:	Copal® G+C	Heraeus Medical GmbH (Germany)	1.0 g gentamicin, 1.0 g clindamycin

Table 2 con.: All tested products and their allocation.

Six standardized cement moulds of each PMMA were produced and provided by Heraeus Medical GmbH (Wehrheim, Germany). The cylindric moulds have a height of 9.9 mm and a diameter of 25 mm. They were coded with capital letters and numbers according to table 3, so that the tests could be performed under blind conditions. The problem in the process was the colour of the cements. Refobacin® Revision and the products from Heraeus Medical GmbH are coloured mint green, while the others are white, so that these moulds could be differed from the rest.

Five cement moulds were exemplary tested for autosterility by laying them into a sterile culture medium for 24 hours and checking the cloudiness of the solution. A sterilisation of the formed bodies with gamma-radiation, hydrogen peroxide gas plasma or ethylene oxid was not performed because of the adulteration of the results. Ethylene oxid for example would have a strong impact on vancomycin. Autoclave sterilization is furthermore not possible because it leads to reduction of the mechanical properties [Münker et al. 2018].

Number	Cement
A1	Palacos® R+G
A2	Simplex® T
A3	Simplex® HVG
A4	Hi-Fatigue® G
A5	CMW™ 1 G
A6	BonOs® R Genta
B1	Copal® G+C
B2	Refobacin® Revision
C1	Simplex® E+C
D1	Copal® G+V
D2	Vancogenx®
E1	Copal® G+C

Table 3: Codification of the tested cements.

2.2 Agar plates

Two different types of self-made agar plates were used for the experiments: Mueller-Hinton agar for MRSA, *Pseudomonas aeruginosa* and E. coli and Mueller-Hinton agar with five percent sheep blood for *Cutibacterium acnes*.

For one litre of Mueller-Hinton agar, 21 g of Mueller-Hinton broth (CM0405 by Oxoid Ltd., Basingstoke, England) and 17 g of agar (05040-250G by Sigma-Aldrich Chemie GmbH, Munich, Germany) were mixed with one litre deionized and sterilized water. The fluid was sterilized and filled in the sterile petri dishes.

Two different sizes of petri dishes were used for the Mueller-Hinton agar in the study: for the first seven days petri dishes with a diameter of 8.5 cm (normal size) which were filled with 20 ml agar and then small dishes with a diameter of 5.5 cm with 7 ml agar were used to lower the ecological damage and the costs.

The blood agar for C. acnes was produced in the same way just with 980 ml water and 20 ml sterilized sheep blood which was added after the sterilization. Because of the big inhibition zones in the first trials only petri dishes in normal size were used for this trial series.

The dried agar plates were spread three times with a bacteria suspension in a 0.5 McFarland turbidity standard. The different cement groups and the bacteria which were tested against the antibiotics are shown in table 4. Group A and D were tested with MRSA and E. coli, group B with MRSA and C. acnes, group C with MRSA and *Pseudomonas aeruginosa* and group E with E. coli.

A six millimeter hole was punched out with a sterile glass pasteur pipette in every agar plate, so that 60 µl of liquid would fit in.

Groups:	Tested bacteria:
Group A with aminoglycosides:	MRSA (ATCC strain 43300) E. coli (ATCC strain 25922)
Group B with gentamicin and clindamycin:	MRSA (ATCC strain 43300) *Cutibacterium acnes* (ATCC strain 11827)
Group C with erythromycin and colistin:	MRSA (ATCC strain 43300) *Pseudomonas aeruginosa* (ATCC strain 27853)
Group D with gentamicin and vancomycin:	MRSA (ATCC strain 43300) E. coli (ATCC strain 25922)
Group E with gentamicin and clindamycin:	E. coli (ATCC strain 25922)

Table 4: The compilation of the cement groups and the bacteria.

2.3 Method

Every individual production step took place under sterile conditions in a sterile workbench.

A phosphate buffered saline solution (PBS) was made with PBS pills from amresco® (Solon, USA) and sterilized. Falcon® tubes with a volume of 50 ml were marked with the specific numbers of the cement moulds and filled with 20 ml PBS. The auto sterile cylinder were taken with a forceps out of the package and put in the tubes. Thereby the forceps were changed for each different cement. The Falcons® were closed and turn upside down, so that the mould was completely covered with the solution. The caps were covered with paraffin wax to improve the solidity. The tubes were stored at room temperature.

After one hour, the cylinders were put out of the buffer solutions with a forceps, dried quickly on a sterile wound compress which lay in a big petri dish and inserted in new prepared Falcon® tubes. The next changes were performed after seven days, 14 days, 21 days, 28 days and 42 days in the same manner. If there was no effect of the antibiotic on the bacterium over two weeks, the cement mould was taken out of the next experiments.
60 µl of each eluate were pipetted in the holes of the prepared agar plates. Three eluates per cement were tested with the same bacterium. The plates with MRSA, E. coli and *Pseudomonas aeruginosa* were incubated by 37 degrees Celsius for 24 hours and those with *Cutibacterium acnes* were stored in an anaerobic bag by 37 degrees Celsius for five days. The measurements of the whole diameter of the bacteria-free areola inclusive the six millimeter hole were performed with a ruler and every zone was saved in pictures. All eluates were stored at -20 degrees Celsius in the fridge.

2.4 Statistics

All statistic calculations were done with IBM® SPSS Statistics 23. Only descriptive and not inductive data analyses were performed because of the following reasons. The first point is the small size of study. Each cement was tested three times which is a too low number to execute variance analyses. Second, big differences in the antibacterial efficacy of the antibiotics were noted, as can be seen in the diagrams, so that a test of significance would not have any additional benefit. Third, small differentials in the inhibiting areolas have no clinical relevance even if they would have a significant p-value below 0.05.
The data set was split into subgroups after antibiotic group (“A” to “E”) and cements (“1” to “6”) to enable divided analysis. Mean and standard deviation were calculated and illustrated in tables and diagrams. All graphs were designed with Microsoft® Excel 2010.

3 Results

The trials showed very different results which are presented separately according to the subgroups (table 2).

3.1 Group A

The experiments with aminoglycosides and methicillin-resistant *Staphylococcus aureus* didn't reach any areola of inhibition.

The detailed data showed that only the Palacos® R+G (A1) was successfully effective over the whole tested time period against E. coli (tables 5 and 6). The trials with CMW™ 1 G were cancelled early after two measurements without inhibiting zone.

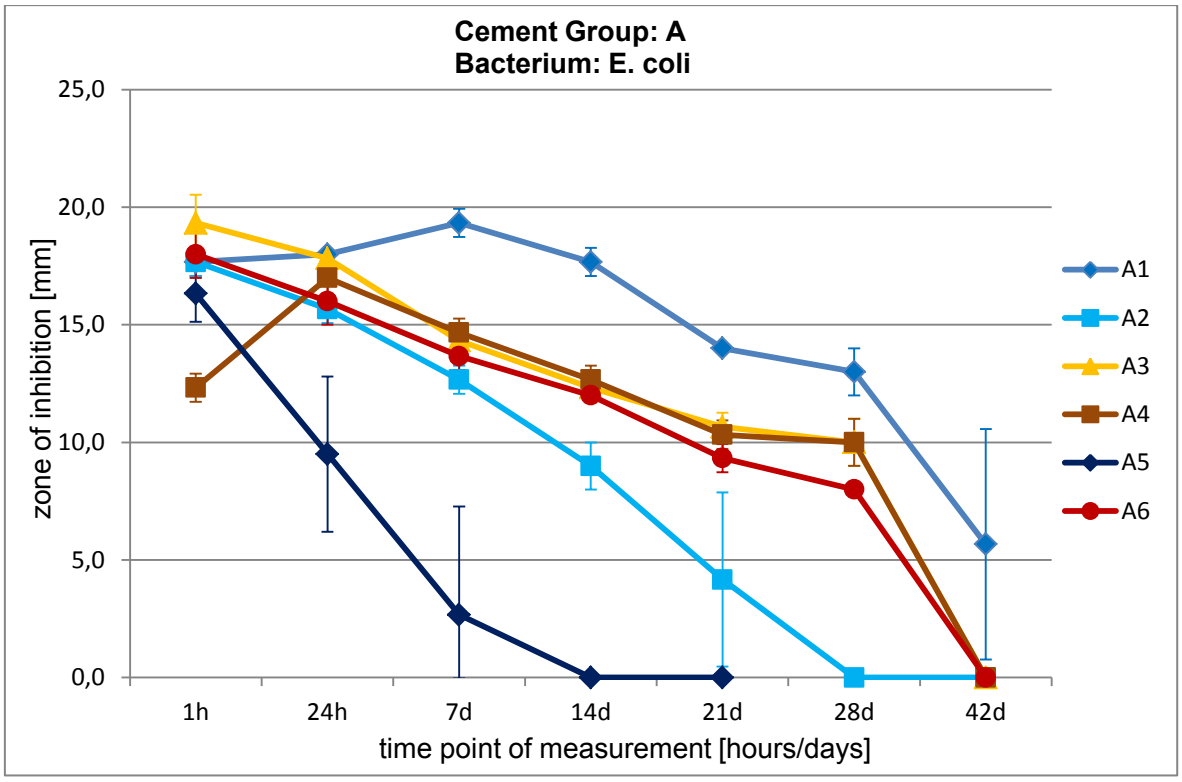
The comparison of the cements (graph 7) permitted to build three different evaluation groups. Palacos® R+G (graph 8) had the best result with a mean of 15.0 mm ± 4.8. Its inhibiting areolas increased to day seven and then gradually declined to 5.7 mm ± 4.9 at the end of the study (table 5). The middle field was built from the cements Simplex® HVG (graph 10), Hi-Fatigue® G (graph 11) and BonOs® R Genta (graph 13) which had a quite similar antibacterial efficacy from 24 hours on with a continuously declining concentration to the 28th day and then no inhibiting areolas. The third group included Simplex® T (graph 9) and CMW™ 1 G (graph 12) which had an early stop of the antibacterial efficacy after seven and 21 days, respectively.

Cement	Experiment	1 h	24 h	7 d	14 d	21 d	28 d	42 d
A1	1	18.0	18.0	19.0	18.0	14.0	13.0	9.0
	2	18.0	18.0	19.0	17.0	14.0	12.0	0
	3	17.0	18.0	20.0	18.0	14.0	14.0	8.0
A2	1	18.0	16.0	13.0	9.0	0	0	0
	2	18.0	16.0	13.0	10.0	7.0	0	0
	3	17.0	15.0	12.0	8.0	5.5	0	0
A3	1	20.0	18.0	14.0	12.0	11.0	11.0	0
	2	18.0	18.0	15.0	13.0	11.0	10.0	0
	3	20.0	17.5	14.0	12.0	10.0	9.0	0
A4	1	12.0	18.0	15.0	13.0	10.0	10.0	0
	2	12.0	17.0	15.0	13.0	11.0	11.0	0
	3	13.0	16.0	14.0	12.0	10.0	9.0	0
A5	1	15.0	13.0	8.0	0	.	.	.
	2	17.0	9.0	0	0	.	.	.
	3	17.0	6.5	0	0	.	.	.
A6	1	17.0	15.0	13.0	12.0	9.0	8.0	0
	2	18.0	17.0	14.0	12.0	10.0	8.0	0
	3	19.0	16.0	14.0	12.0	9.0	8.0	0

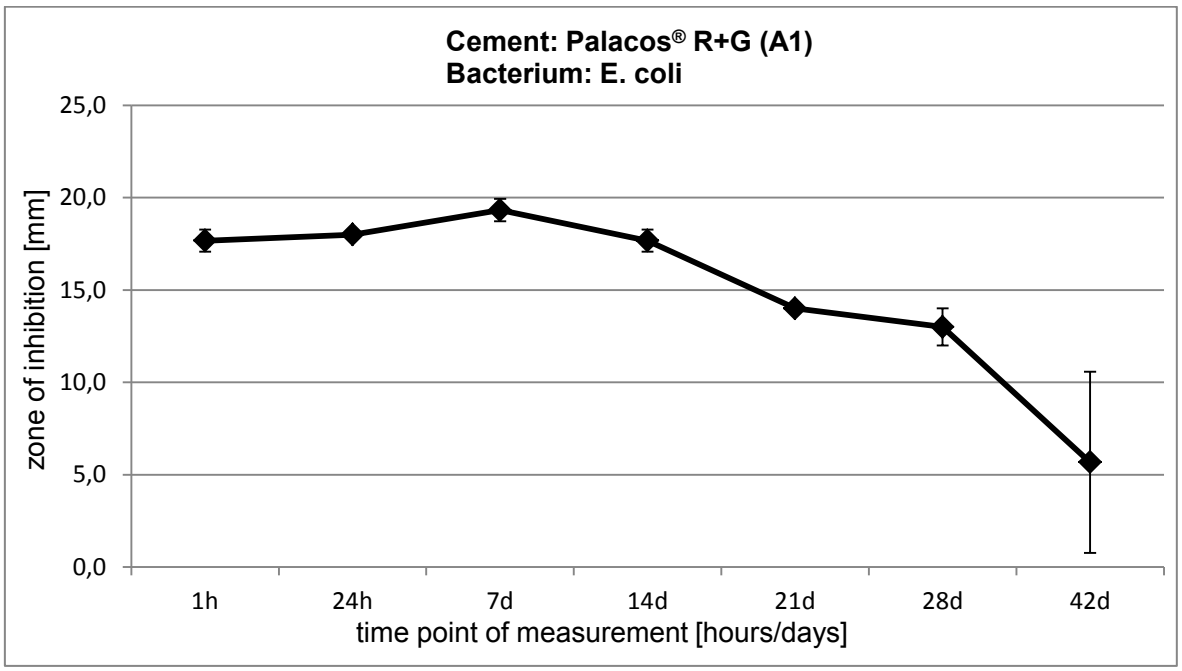
Table 5: Measured inhibiting zones of the six cements of group A which were tested with *Escherichia coli*. All data in millimeter. A1: Palacos® R+G; A2: Simplex® T; A3: Simplex® HVG; A4: Hi-Fatigue® G; A5: CMW™ 1 G; A6: BonOs® R Genta.

Cement	1 h	24 h	7 d	14 d	21 d	28 d	42 d
A1	17.7 ± 0.6	18.0 ± 0	19.3 ± 0.6	17.7 ± 0.6	14.0 ± 0	13.0 ± 1.0	5.7 ± 4.9
A2	17.7 ± 0.6	15.7 ± 0.6	12.7 ± 0.6	9.0 ± 1.0	4.2 ± 3.7	0 ± 0	0 ± 0
A3	19.3 ± 1.2	17.8 ± 0.3	14.3 ± 0.6	12.3 ± 0.6	10.7 ± 0.6	10.0 ± 1.0	0 ± 0
A4	12.3 ± 0.6	17.0 ± 1.0	14.7 ± 0.6	12.7 ± 0.6	10.3 ± 0.6	10.0 ± 1.0	0 ± 0
A5	16.3 ± 1.2	9.5 ± 3.3	2.7 ± 4.6	0 ± 0	.	.	.
A6	18.0 ± 1.0	16.0 ± 1.0	13.7 ± 0.6	12.0 ± 0	9.3 ± 0.6	8.0 ± 0	0 ± 0

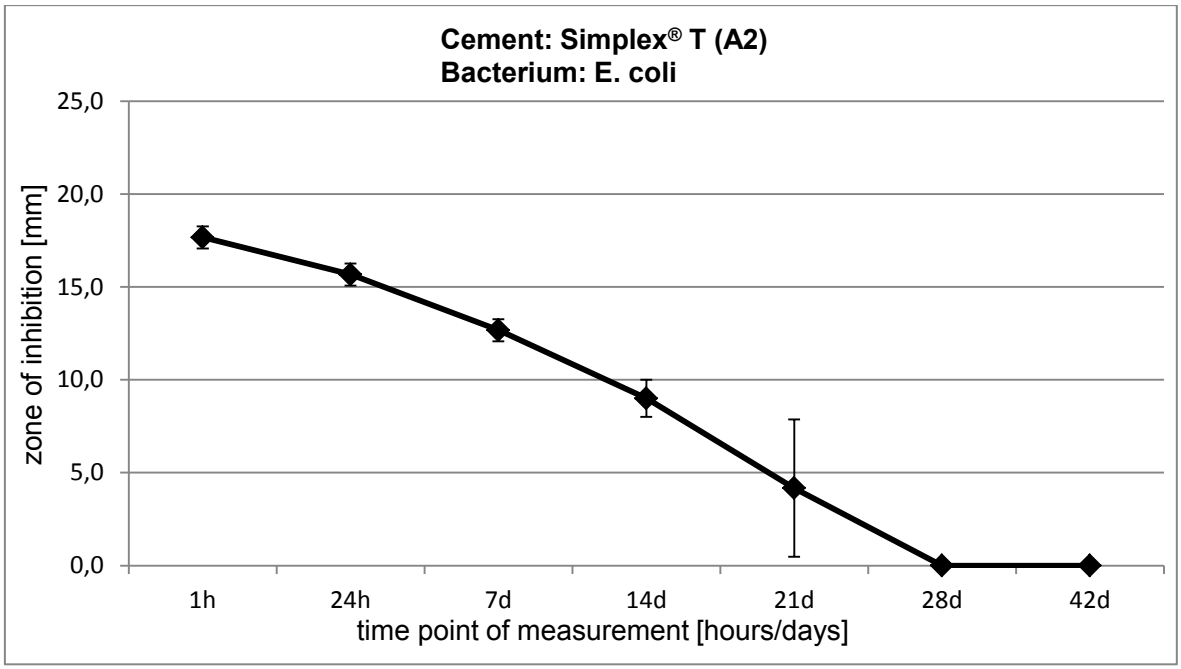
Table 6: Calculated mean and standard deviation of the group A cements which were tested with E. coli. All data in millimeter. A1: Palacos® R+G; A2: Simplex® T; A3: Simplex® HVG; A4: Hi-Fatigue® G; A5: CMW™ 1 G; A6: BonOs® R Genta.



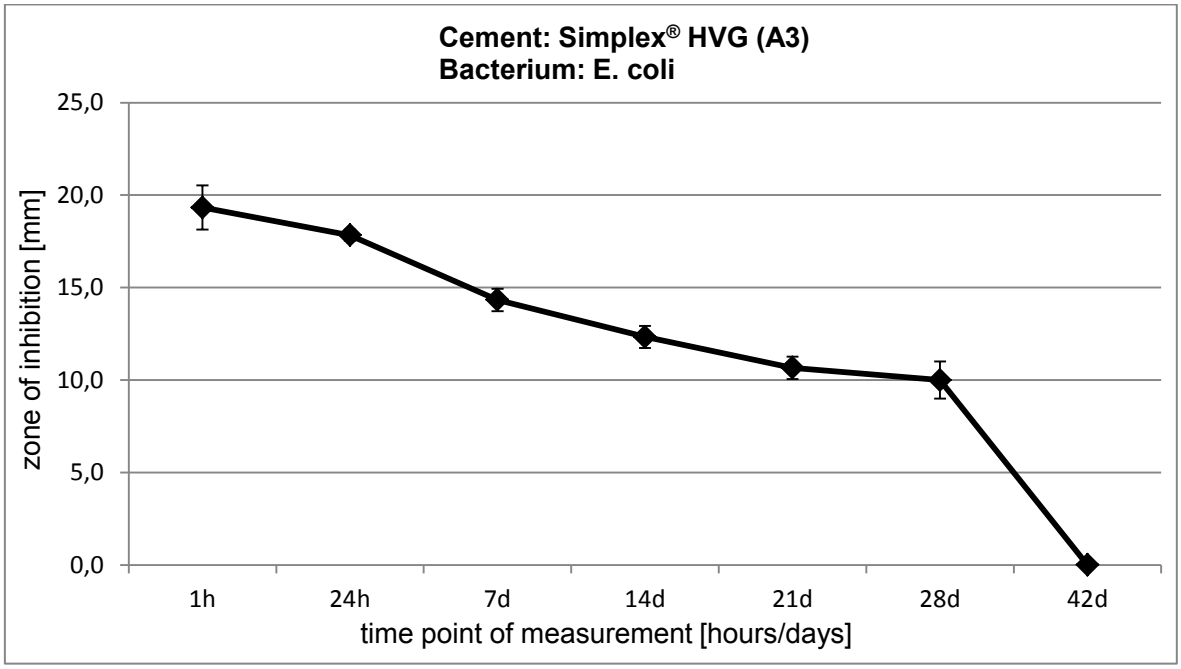
Graph 7: Mean zones of inhibition and standard deviation of all six cements of group A tested with E. coli. A1: Palacos® R+G; A2: Simplex® T; A3: Simplex® HVG; A4: Hi-Fatigue® G; A5: CMW™ 1 G; A6: BonOs® R Genta.



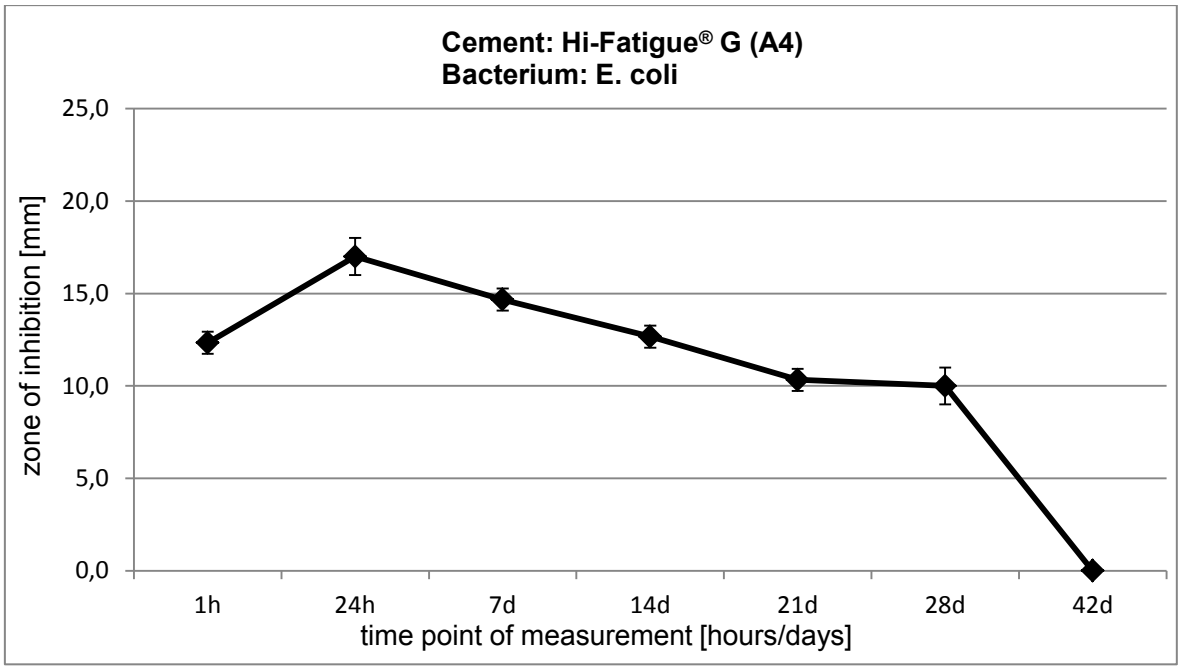
Graph 8: Mean inhibiting areolas and standard deviation of Palacos® R+G (A1) tested with E. coli.



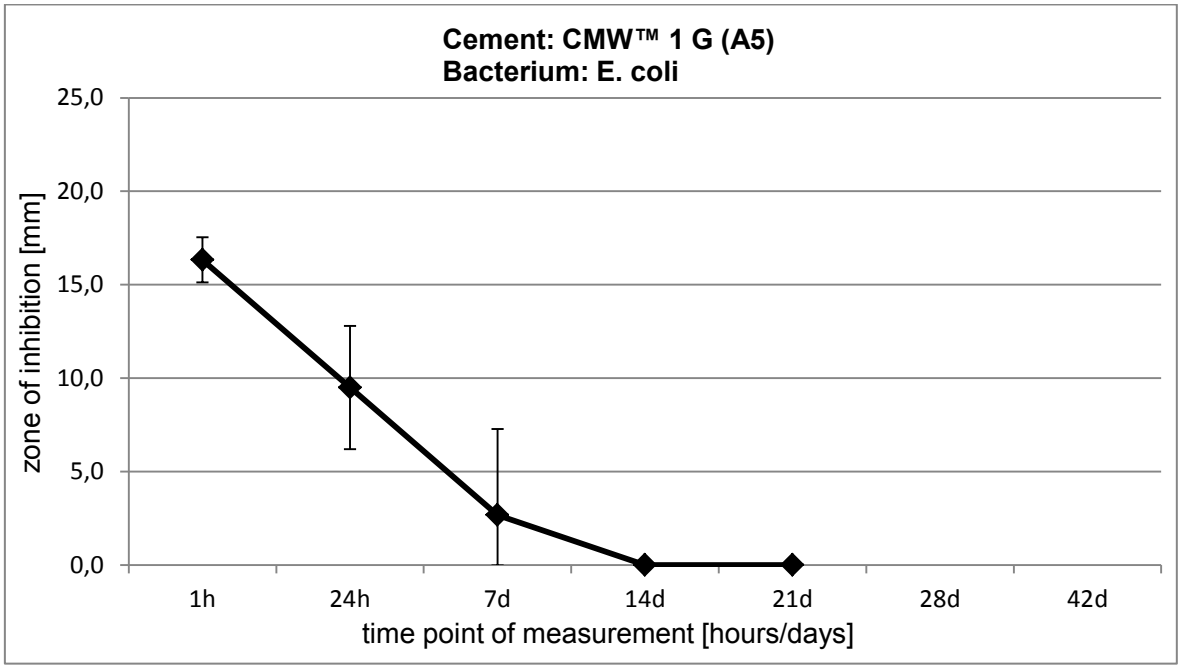
Graph 9: Mean zones of inhibition and standard deviation of Simplex® T (A2) tested with E. coli.



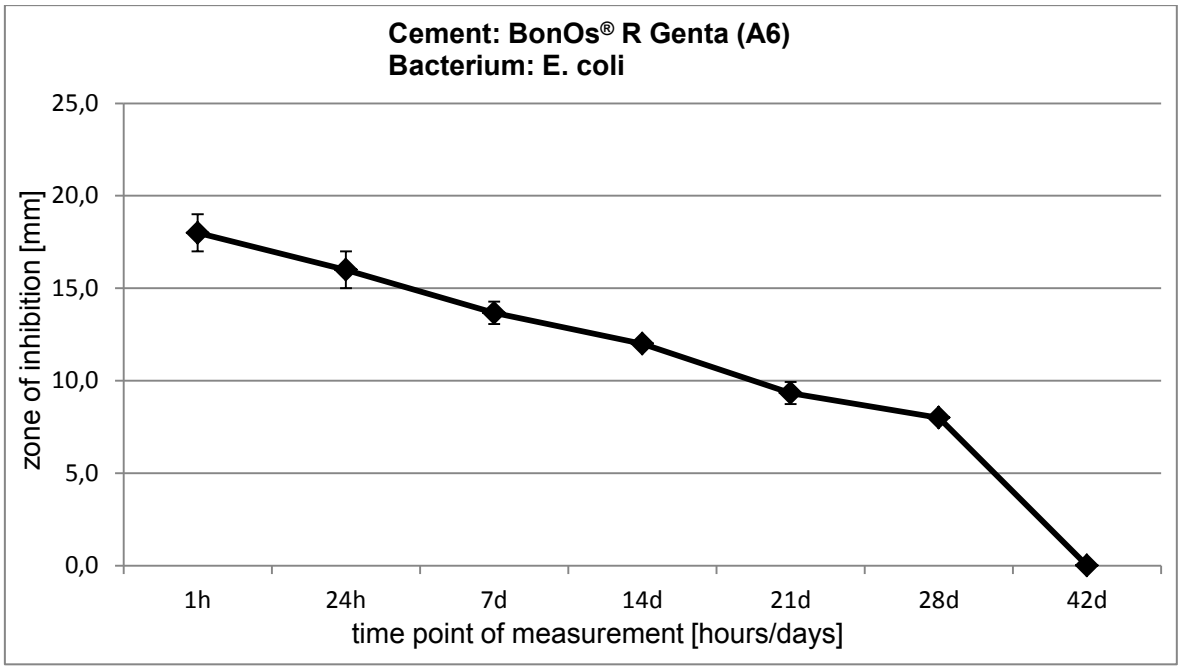
Graph 10: Mean inhibiting areolas and standard deviation of Simplex® HVG (A3) tested with E. coli.



Graph 11: Mean zones of inhibition and standard deviation of Hi-Fatigue® G (A4) tested with E. coli.



Graph 12: Mean inhibiting areolas and standard deviation of CMW™ 1 G (A5) tested with E. coli.



Graph 13: Mean zones of inhibition and standard deviation of BonOs® R Genta (A6) tested with E. coli.

3.2 Group B

No trial with the cements containing gentamicin and clindamycin which were tested with methicillin-resistant *Staphylococcus aureus* achieved any zone of inhibition.

The agar plates with C. acnes were each measured three times, because the inhibiting zones were oval and not round. The calculated means are shown in table 7. In total, the areolas of inhibition were about three times larger in comparison to all other experiments.

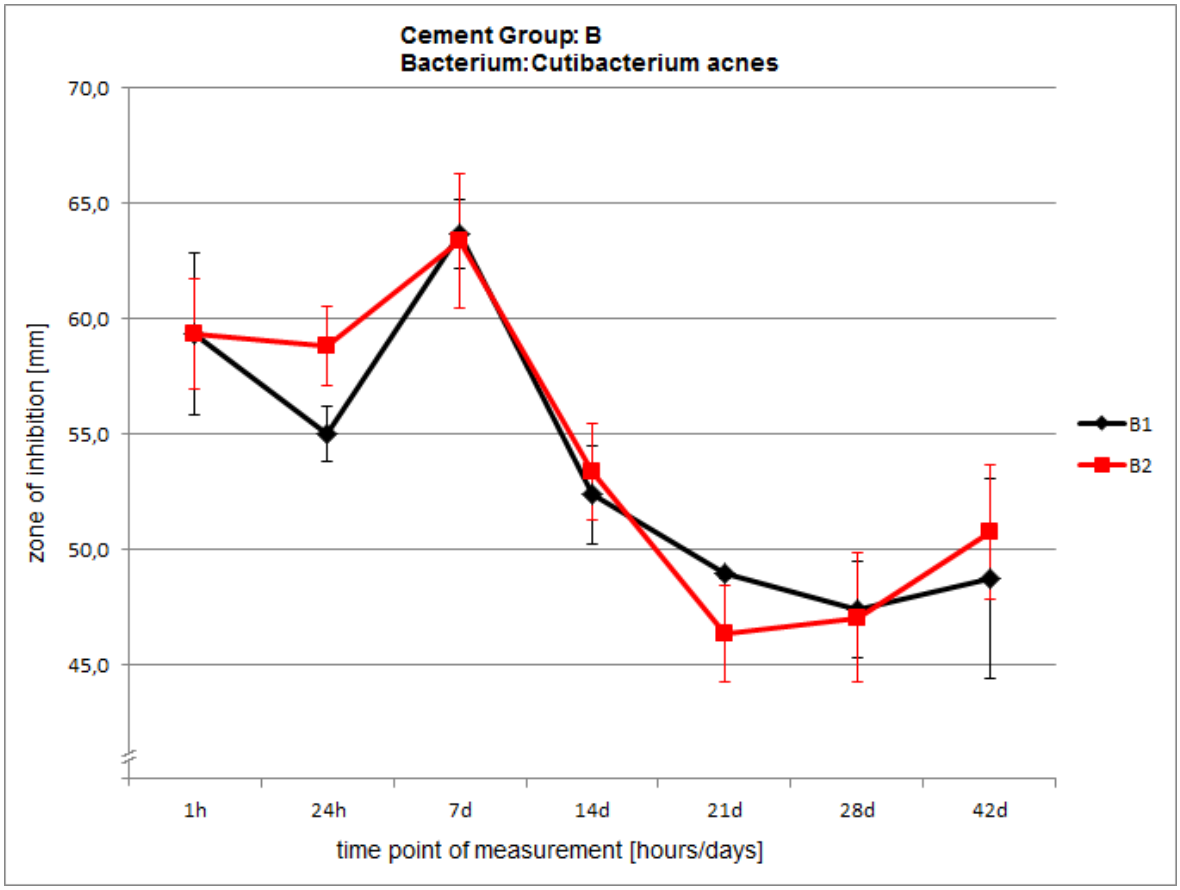
Cement	Experiment	1 h	24 h	7 d	14 d	21 d	28 d	42 d
B1	1	63.0	56.3	65.0	54.0	48.6	49.0	49.6
	2	59.0	54.6	64.0	50.0	49.0	45.0	44.0
	3	56.0	54.0	62.0	53.0	49.0	48.0	52.5
B2	1	56.6	60.6	65.0	54.0	48.0	49.5	48.5
	2	61.0	58.6	65.0	55.0	47.0	47.5	54.0
	3	60.3	57.3	60.0	51.0	44.0	44.0	49.6

Table 7: Mean zones of inhibition of the group B cements calculated from three measurements per agar plate. All data in millimeter. B1: Copal® G+C; B2: Refobacin® Revision

Cement	1 h	24 h	7 d	14 d	21 d	28 d	42 d
B1	59.3 ± 3.5	55.0 ± 1.2	63.7 ± 1.5	52.3 ± 2.1	48.9 ± 0.2	47.3 ± 2.1	48.7 ± 4.3
B2	59.3 ± 2.4	58.8 ± 1.7	63.3 ± 2.9	53.3 ± 2.1	46.3 ± 2.1	47.0 ± 2.8	50.7 ± 2.9

Table 8: Mean and standard deviation of all zones of inhibition of the group B cements which were tested with C. acnes. All data in millimeter. B1: Copal® G+C; B2: Refobacin® Revision

The graph 14 allowed a simple comparison of both cements. The zones of inhibition were quite similar over the whole six weeks, except of the measurement after 24 hours which showed a small difference, where Copal® G+C (B1) achieved a inhibiting zone of 55.0 mm ± 1.2 and Refobacin® Revision (B2) 58.8 mm ± 1.7 (table 8). The areolas of inhibition increased from day 28 to 42 because of the longer time period from one to two weeks.



Graph 14: Measured inhibiting areolas of the group B cements which were tested with *Cutibacterium acnes*. B1: Copal® G+C; B2: Refobacin® Revision.

3.3 Group C

No inhibiting areolas could be measured by the experiments with Simplex® E+C (C1) which was tested with MRSA and *Pseudomonas aeruginosa*.

3.4 Group D

The fourth group compared the cements containing gentamicin and vancomycin. The Copal® G+V (D1) did clearly better in both test series than Vancogenx® (D2).

3.4.1 Tests with E. coli

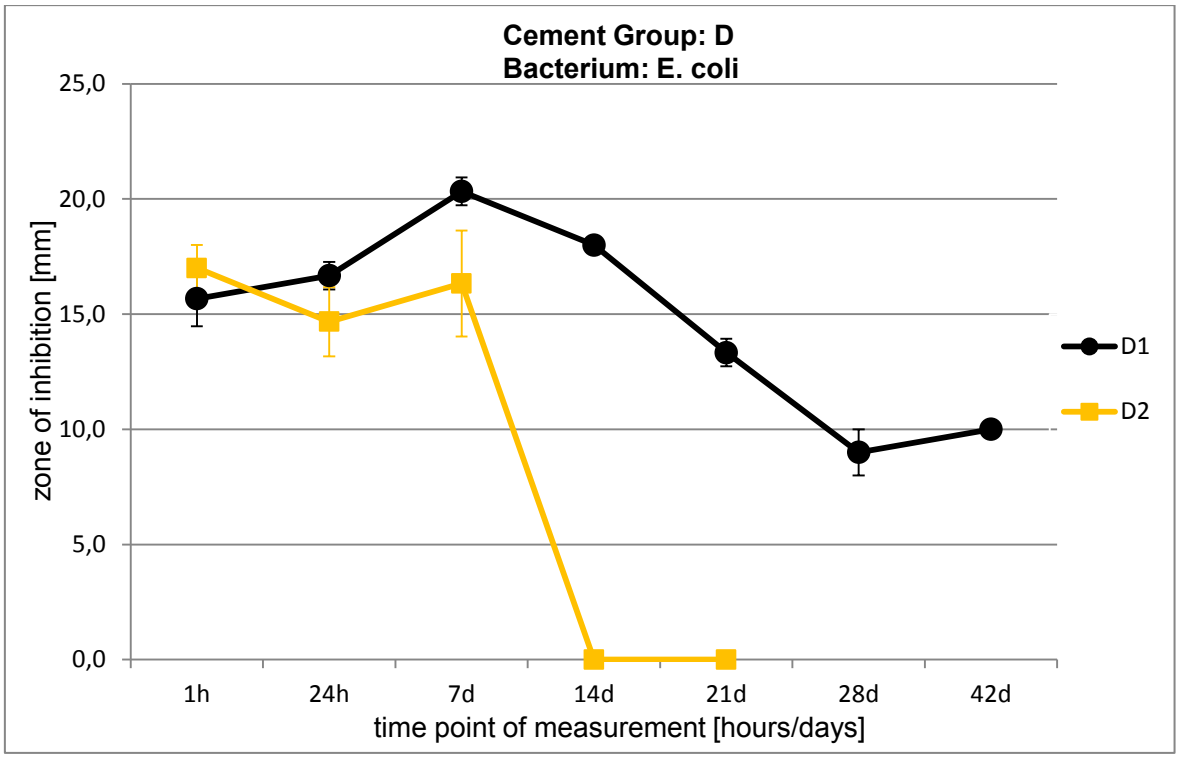
The Copal® G+V (D1) had a good antibacterial efficacy over the whole experimental time with a peak of 20.3 mm ± 0.6 at the third measurement and a minimum of 9.0 mm ± 1.0 at day 28 (table 9 and 10). The areolas of inhibition increased from day 28 to 42 because of the longer time period from one to two weeks. The Vancogenx® (D2) had only zones of inhibition over the first seven days which were comparable with D1. After that there was no zone of inhibition measurable, so that the trial series was cancelled at day 21 after two measurements without inhibiting areola. The graph 15 figures the big difference between the PMMAs.

Cement	Experiment	1 h	24 h	7 d	14 d	21 d	28 d	42 d
D1	1	15.0	17.0	20.0	18.0	13.0	9.0	10.0
	2	17.0	17.0	21.0	18.0	14.0	10.0	10.0
	3	15.0	16.0	20.0	18.0	13.0	8.0	10.0
D2	1	18.0	16.0	15.0	0	0	.	.
	2	17.0	15.0	19.0	0	0	.	.
	3	16.0	13.0	15.0	0	0	.	.

Table 9: All detailed measurements of the group D cements which were tested with E. coli. All data in millimeter. D1: Copal® G+V; D2: Vancogenx®.

Cement	1 h	24 h	7 d	14 d	21 d	28 d	42 d
D1	15.7 ± 1.2	16.7 ± 0.6	20.3 ± 0.6	18.0 ± 0	13.3 ± 0.6	9.0 ± 1.0	10.0 ± 0
D2	17.0 ± 1.0	14.7 ± 1.5	16.3 ± 2.3	0 ± 0	0 ± 0	.	.

Table 10: Calculated mean and standard deviation of group D which was tested with E. coli. All data in millimeter. D1: Copal® G+V; D2: Vancogenx®.



Graph 15: Mean zones of inhibition and standard deviation of both cements of group D measured with E. coli. D1: Copal® G+V; D2: Vancogenx®.

3.4.2 Tests with MRSA

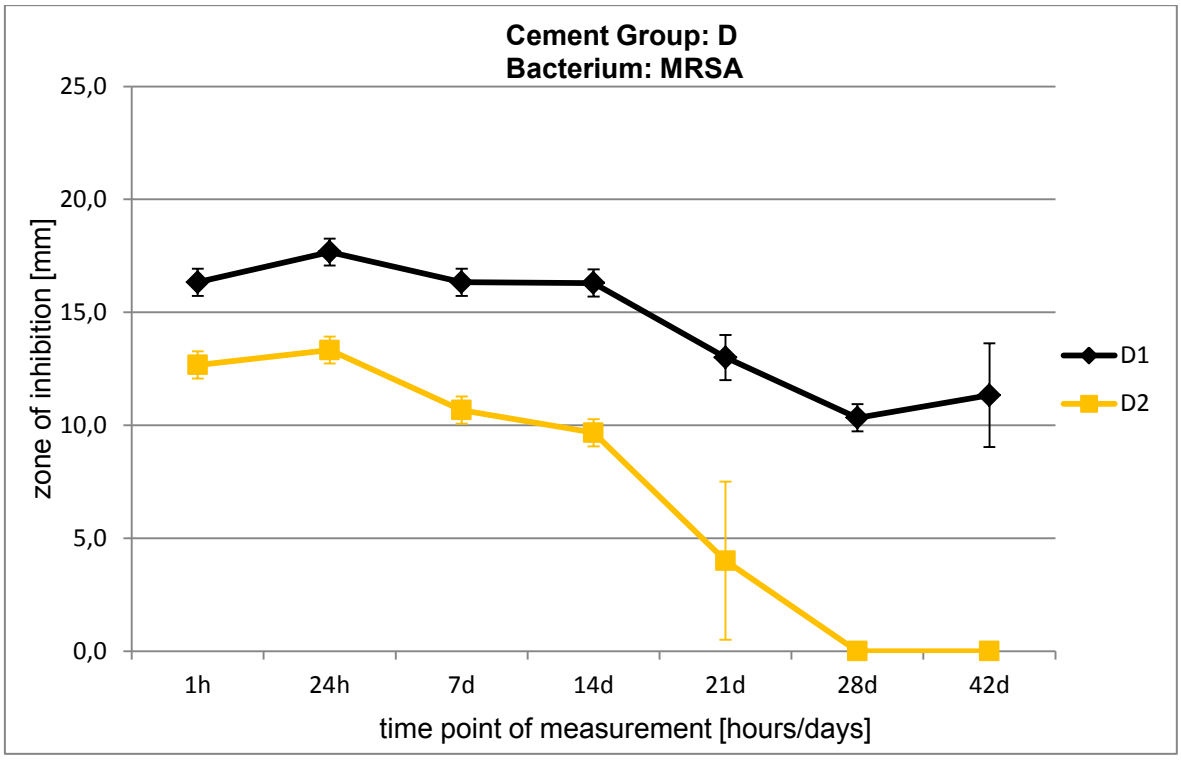
Copal® G+V (D1) had a good antibacterial efficacy over six weeks with a slight fall at the second half. It achieved a mean of 14.5 mm ± 2.9 (table 11 and 12). Vancogenx® (D2) had measurable zones of inhibition from the beginning to day 21 and therewith a better result than in the experiments against E. coli. There was no adequate zone of inhibition later in the study. The comparison of both cements showed that the cement D2 had always lower inhibiting areolas then D1 (graph 16).

Cement	Experiment	1 h	24 h	7 d	14 d	21 d	28 d	42 d
D1	1	17.0	18.0	17.0	17.0	13.0	10.0	10.0
	2	16.0	17.0	16.0	16.0	12.0	10.0	10.0
	3	16.0	18.0	16.0	16.0	14.0	11.0	14.0
D2	1	13.0	13.0	11.0	9.0	0	0	.
	2	13.0	14.0	11.0	10.0	6.0	0	0
	3	12.0	13.0	10.0	10.0	6.0	0	0

Table 11: Measured inhibiting zones of the two cements of group D which were tested with MRSA. All data in millimeter. D1: Copal® G+V; D2: Vancogenx®.

Cement	1 h	24 h	7 d	14 d	21 d	28 d	42 d
D1	16.3 ± 0.6	17.7 ± 0.6	16.3 ± 0.6	16.3 ± 0.6	13.0 ± 1.0	10.3 ± 0.6	11.3 ± 2.3
D2	12.7 ± 0.6	13.3 ± 0.6	10.7 ± 0.6	9.7 ± 0.6	4.0 ± 3.5	0 ± 0	0 ± 0

Table 12: Calculated mean and standard deviation of the group D cements which were tested with MRSA. All data in millimeter. D1: Copal® G+V; D2: Vancogenx®.



Graph 16: Measured inhibiting zones of the group D cements which were tested with MRSA. D1: Copal® G+V; D2: Vancogenx®.

3.5 Group E

Copal® G+C was tested with *Escherichia coli* and achieved a large inhibiting zone over the whole time of the experiment with a mean of 17.7 mm ± 2.9 (table 13 and 14). The antibacterial efficacy is identical to the trials with *Cutibacterium acnes* (compare cement B1 in graph 20 and 21 with graph 14 and 15).

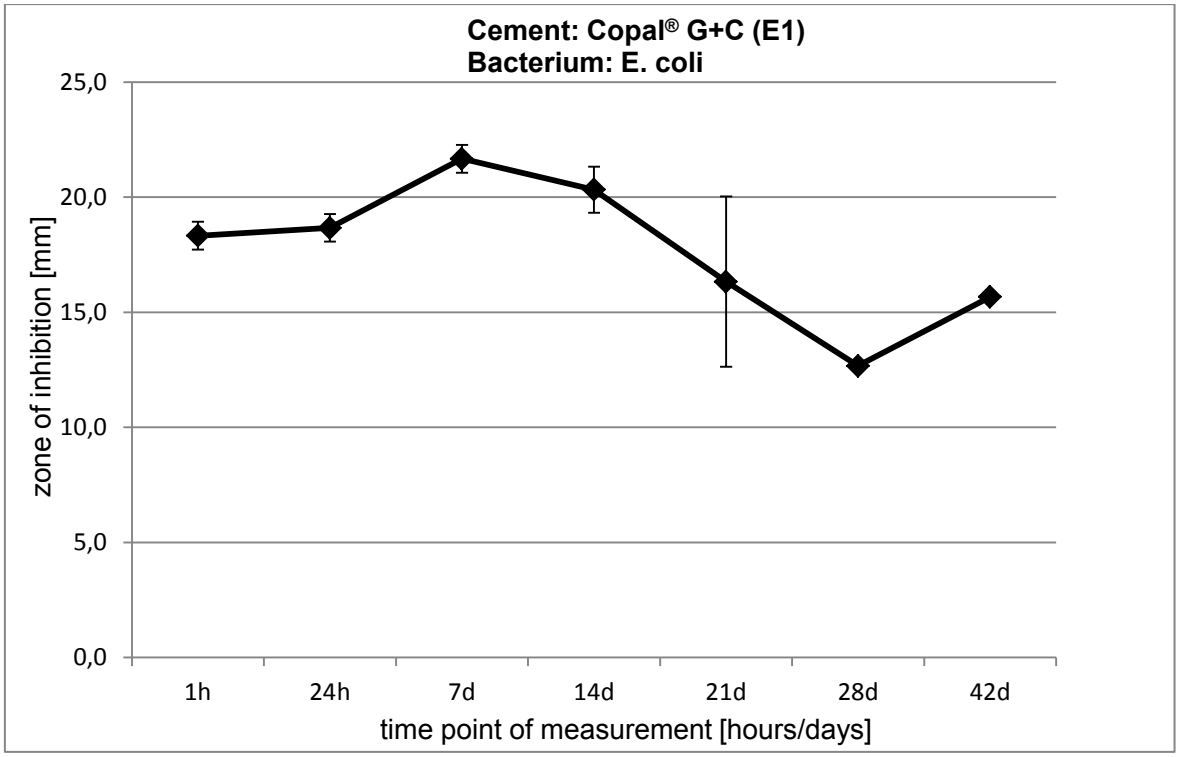
Cement	Experiment	1 h	24 h	7 d	14 d	21 d	28 d	42 d
E1	1	18.0	19.0	22.0	20.0	16.0	12.0	17.0
	2	19.0	18.0	22.0	20.0	16.0	13.0	15.0
	3	18.0	19.0	21.0	21.0	17.0	13.0	15.0

Table 13: Measured zones of inhibition of Copal® G+C (E1) with E. coli. All data in millimeter.

Cement	1 h	24 h	7 d	14 d	21 d	28 d	42 d
E1	18.3 ± 0.6	18.7 ± 0.6	21.7 ± 0.6	20.3 ± 0.6	16.3 ± 0.6	12.7 ± 0.6	15.7 ± 1.2

Table 14: Calculated mean and standard deviation of all zones of inhibition of Copal® G+C (E1) which was tested with E. coli. All data in millimeter.

The antibacterial efficacy is shown in the graphs 20 and 21. The inhibiting areolas increased to day 7, then gradually declined to 12.7 mm ± 0.6 at week four and increased again to 15.7 mm ± 1.2 (table 14). The areolas of inhibition increased from the sixth measurement to the seventh, because of the longer time period from one to two weeks.



Graph 17: Mean zones of inhibition and standard deviation of Copal® G+C (E1) measured with E. coli.

4 Discussion

In total, only four cements showed a relevant antibacterial efficacy over 42 days: Palacos® R+G (A1), Refobacin® Revision (B2), Copal® G+C (B1, E1) and Copal® G+V (D1). Next to the definition of Malhotra et al. [2018], Palacos® R+G is thereby the only cement which is no revision bone cement, because it contains only one antibiotic in a too low concentration.

4.1 MRSA

The experiments with methicillin-resistant *Staphylococcus aureus* (MRSA) were negative with nine out of eleven cements. Microbial sensitivity tests showed that the used strain (ATCC 43300) is resistant to gentamicin and clindamycin and sensitive to vancomycin (table 15). Erythromycin is also tested as ineffective, although Amin et al. [2015] describe large zones of inhibition. Colistin was not tested, because all MRSA strains have a natural resistance. Simplex® E+C could not achieve any effect which could be caused by resistance of the bacterial strain or an insufficient elution of the antibiotic combined with an inadequate concentration in the cement powder. Only the vancomycin containing cements of group D were successful against this MRSA strain.

Between 2012 and 2016 MRSA had a resistance rate against gentamicin of 5.0 to 6.6 percent in Germany. Erythromycin was in the same time period in 56.3 to 65.7 percent ineffective and clindamycin in 47.0 to 58.8 percent with downward tendency in each case [Robert Koch Institute 2018].

Hence the safest combination of local antibiotics for the treatment of MRSA is gentamicin with vancomycin. It is well known that the combination of gentamicin and vancomycin is characterized in a strong physical and pharmaceutical synergism [Bertazzoni Minelli et al. 2011; Kühn et al. 2017; Mulazimoglu et al. 1996].

Antibiotic	Minimum inhibitory concentration (MIC)	Interpretation
Gentamicin	>= 16	Resistant
Tobramycin		Resistant
Erythromycin	>= 8	Resistant
Inducible Clindamycin resistance	Negative	Negative
Clindamycin	>= 4	Resistant
Vancomycin	1	Sensitive

Table 15: Extract of the microbial sensitivity tests with MRSA strain ATCC 43300. The whole trial is listed in the annexe.

Group D compared two polymethylmethacrylates with gentamicin and vancomycin: Copal® G+V and Vancogenx®. While the tests against E. coli showed the effect of gentamicin, the tests against MRSA outlined the effect of vancomycin, because each bacterium has a resistance to the respective other antibiotic. Nevertheless, the combined admixture of both antibiotics in the bone cement is recommendable, because the antibiotics have a synergistic effect on each other in elution and efficacy [Bertazzoni Minelli et al. 2011; Kühn et al. 2017; Mulazimoglu et al. 1996].

The tests showed that Copal® G+V reached a better effect over the whole tested time period. The primary reason could be the better elution of gentamicin despite the lower concentration (0.5 g versus 1 g; graph 15) and of secondary importance the higher vancomycin concentration (1 g versus 2 g).

4.2 Pseudomonas aeruginosa

According to Fass and Barnishan [1979] and the EUCAST susceptibility breakpoints, the used strain of *Pseudomonas aeruginosa* (ATCC 27853) is resistant to erythromycin and has an intermediate sensibility to colistin with a minimum inhibitory concentration (MIC) of 2-4 µg/ml. However, in this context it must be remembered that the EUCAST susceptibility breakpoints apply for the systemic use of antibiotics. A local antibiotic treatment can reach higher concentrations than a systemic application, so that the breakpoints are not directly transferable [Kühn et al. 2017].

Hence, the missing inhibition areola of Simplex® E+C cannot be fully explained. Because of the additional negative tests with MRSA, an insufficient elution and too low concentrations of antibiotics in the cement could be possible.
Scott and Higham [2003] showed that the use of PMMA with aminoglycosides is mostly adequate for a sufficient treatment of an infection with *Pseudomonas aeruginosa*. Simplex® T failed only in three and Palacos® R+G in seven out of 100 clinical isolates.

4.3 Comparison with other studies

Palacos®, CMW™ and Simplex® are old fashion cements which have been available for many decades. This is the reason for having many comparative studies. A research in PubMed yielded 34 studies. In conclusion, 13 studies described a well elution or antibacterial efficacy of the Palacos® cement [Armstrong et al. 2002; Boelch et al. 2017; Dall et al. 2007; Elson et al. 1977; Gallo et al. 2013; Hoff et al. 1981; Holm and Vejlsgaard 1976; Miola et al. 2013; Neut et al. 2010; Van De Belt et al. 2000; Wahlig and Buchholz 1972; Wahlig and Dendeldein 1980; Wannske et al. 1976], while four trials reported a release or antibacterial efficacy over shorter time periods (table 16) [Al Thaher et al. 2018; Ensing et al. 2008; Moojen et al. 2008; Picknell et al. 1979]. Table 17 shows five studies which tested a long-term elution or antibacterial efficacy of CMW™, two of which reached similar results [Bayston and Milner 1982; Van De Belt et al. 2000] and three described a longer release or antibacterial efficacy [Armstrong et al. 2002; Holm and Vejlsgaard 1976; Torrado et al. 2001]. Table 18 shows the conclusion of previous tests with Simplex® T of which two received better results [Karek et al. 2017] and two worse than in this study [DiCicco et al. 2003; Klekamp et al. 1999; Moojen et al. 2008; Stevens et al. 2005].

Palacos® R+G				
Reference	**Conclusion**	**Production**	**Measurement**	**Funding**
Corresponding to the results:				
Armstrong et al. 2002	Antibiotic release over 21 days.	Cement blocks (5x1x0.4 cm) were produced with the Optivac vacuum mix system (Schering-Plough) and put in 20 ml normal saline which was changed after each time period.	Fluorescence polarization immunoassay (Abbot TDx system, Abbot Laboratories, Chicago, USA).	Unknown.
Boelch et al. 2017	Elution over six weeks.	PMMA was mixed under atmospheric pressure and pressed in discs (15x5 mm) which were put in 2 ml fetal calf serum over six weeks with serum change after each measurement point.	Clinical analyzer with homogene enzyme immunoassay (Hitachi Analyzer, Roche, Mann-heim, Germany)	Internal sources of the Department of Orthopaedic Surgery, Koenig-Ludwig-Haus.
Dall et al. 2007	Good release over the whole test period of three days.	Bone cement was vacuum mixed with the Easymix cement injector (Coripharm GmbH & Co KG, Dieburg, Germany). Discs (25 mm diameter, 10 mm high) were pressed and placed in 20 ml phosphate buffer solution which was changed after every time period.	Fluorescence polarization immunoassay (Abbot TDx system, Abbott Park, Illinois, USA).	None.

Table 16: Conclusion of previous tests with Palacos® bone cement.

Elson et al. 1977	Palacos® has an antibacterial efficacy over 24 days.	Plain Palacos® bone cement was manually mixed with 0.5 g or 1.0 g gentamicin under atmospheric pressure and pressed in moulds (2x2x0.5 cm or 2x2x1 cm) which were placed in distilled water with change after every measurement.	Standard plate diffusion method.	Messrs E. Merck (Darmstadt, West Germany).
Gallo et al. 2013	Elution over at least eight days.	Vacuum mixed PMMA was pressed in moulds (5x3 mm) and put in 1 ml saline and 1 ml Müller-Hinton medium which was changed after every time period.	Liquid chromatograph (Prominence LC20, Shimada-zu, Kyoto, Japan).	Unknown.
Hoff et al. 1981	Palacos® releases gentamicin over six weeks.	Plain Palacos® PMMA was manually mixed with 2 g gentamicin with a ^{3}H isotope label under atmospheric pressure. Pellets (unknown size) were placed in 10 ml distilled water and changed after every measurement for six weeks.	Isotopic assay with the Packard tri-carb liquid scintillation counter and agar diffusion assay with *Staphylococcus epidermidis*.	Schering Corporation.
Holm and Vejlsgaard 1976	Palacos® has an antibacterial efficacy over more than 90 days.	Plain bone cement was manually mixed with 0.5 g gentamicin under atmospheric pressure and pressed in moulds (10x4x110 mm). These were incubated in human serum which was changed every day.	Inhibition zone assay with 0.2 ml serum on a 6 mm paper disc.	Unknown.

Table 16 con.: Conclusion of previous tests with Palacos® bone cement.

Miola et al. 2013	Good release over 28 days.	Palacos® R+G was mixed under atmospheric pressure and pressed in cylinders (10x5 mm) which were set in 30 ml simulated body fluid. 2 ml were periodically spiked for analysis.	High pressure liquid chromatography technique.	Unknown.
Neut et al. 2010	Good elution over seven days.	An interfacial gap model (200 mg) with gap volume of 6 µl was produced out of bone cement which was mixed under atmospheric pressure. First test: Different tests were done with 6 µL or 10 ml phosphate buffered saline without changes. 500 µl samples were taken after each time period or the 6 µl were aspirated out of the gaps. Second test: A suspension with bacteria was put in the gap and serial dilutions were poured on TSB agar plates.	Fluorescence spectro-photometer (Spectronic® 20 Genesys™; Spectronic Instruments, Rochester, New York, USA).	University Medical Center Groningen (The Netherlands), DePuy Inter-national Ltd. (United Kingdom).

Table 16 con.: Conclusion of previous tests with Palacos® bone cement.

Van De Belt et al. 2000	Good elution profile over one week. 8.4% ± 0.4 total release over one week.	PMMA was mixed under atmospheric pressure and pressed in moulds (6x3.2 mm) which were put in 10 ml PBS. The solution was changed after every measurement.	Fluorescence polarization immunoassay (Abbott AxSym; Abbott Laboratories, Abbott Park, Illinois, USA).	Unknown.
Wahlig and Buchholz 1972	Palacos® releases gentamicin over more than a year.	Plain Palacos® bone cement was manually mixed with gentamicin under atmospheric pressure.	Unknown.	Unknown.
Wahlig and Dendeldein 1980	Good antibacterial efficacy over 10 days.	Plain Palacos® PMMA was manually mixed with gentamicin under atmospheric pressure and pressed in blocks (25x3 mm, 25x10 mm or 25x20 mm). The blocks were placed in 20 ml PBS or human serum which was changed after every measurement.	Agar diffusion test with paper disc method with *Bacillus subtilis* and calibration curve with standard dilutions.	E. Merck, Darmstadt, Germany
Wannske et al. 1976	Palacos® has an antibacterial efficacy over 10 month.	Plain Palacos® bone cement was manually mixed with 250 mg gentamicin under atmospheric pressure and placed in 24 tibias of sheep which were previously infected with *Staphylococcus aureus*. After some time each tibial corticalis was taken out, crushed and inserted in buffer solution for analysis.	Agar diffusion tests with calibration curve with standard dilutions.	Unknown.

Table 16 con.: Conclusion of previous tests with Palacos® bone cement.

Deviate from the results:				
Al Thaher et al. 2018	Elution only over 10 days in the fluorescence spectroscopy, but antimicrobial effectiveness up to 21 days with *Staphylococcus epidermidis*).	Palacos© R+G was mixed under atmospheric pressure and pressed in moulds (6x10 mm) which were put in 3 ml PBS. The solution was changed after every time period.	Fluorescence spectroscopy (FLUOROstar Optina, BMG Labtech) and agar diffusion assay with different bacteria.	None.
Ensing et al. 2008	Release only over some hours in the immunoassay and antibacterial efficacy over 3 days.	Bone cement was mixed under atmospheric pressure and pressed in moulds (6x3 mm). The blocks were set in 20 ml PBS. After each time period 0.5 ml were taken out for analysis.	Fluorescence polarization immunoassay (AxSYM; Abbott Laboratories, Abbott Park, Illinois, USA) and inhibition zone assay.	None.
Moojen et al. 2008	Palacos® has a relevant elution of gentamicin over the first three days and on the sixth day. Then up to 42 days no elution.	PMMA was mixed under atmospheric pressure and pressed in hip spacers (9x125 mm stem size, 51 mm head diameter). The spacer was placed in 1 l PBS from which 250 µl were periodically taken out for analysis.	Fluorescent polarizing immunoassay (AxSym system, Abbott Diagnostics, Hoofddorp, The Netherlands).	Biomet (The Netherlands), Stryker (Benelux).

Table 16 con.: Conclusion of previous tests with Palacos® bone cement.

Picknell et al. 1979	Palacos® shows an antibacterial efficacy only over two days.	Plain Palacos© was manually mixed with 1 g gentamicin under atmospheric pressure and pressed in cylinders (7 mm high, 7 mm diameter). The cylinders were set in 10 ml sterile water.	Agar diffusion micro-biology assay with *Bacillus subtilis*.	Unknown.

Table 16 con.: Conclusion of previous tests with Palacos® bone cement.

CMW™ 1 G				
Reference	**Conclusion**	**Production**	**Measurement**	**Funding**
Corresponding to the results:				
Bayston and Milner 1982	Antibacterial efficacy only over eight days.	Plain CMW™ 1 bone cement was manually mixed with 0.4 g gentamicin under atmospheric pressure and pressed in moulds (1 mm high, 20 mm diameter) which were planted on the agar plate for 24 hours and then put on a new plate.	Serial plate transfer method with diagnostic sensitivity test agar plates with *Staphylococcus albus*.	Unknown.
Van De Belt et al. 2000	Release over seven days with 5.3% ± 0.2 total elution of gentamicin.	PMMA was mixed under atmospheric pressure and pressed in discs (6 mm diameter, 100 mg) which were put in 10 ml PBS. After each measurement, the solution was changed.	Fluorescence polarization immunoassay (Abbott AxSym; Abbott Laboratories, Abbott Park, Illinois, USA).	Unknown.

Table 17: Conclusion of previous studies of CMW™ 1 G.

Deviate from the results:				
Armstrong et al. 2002	Antibiotic release over 21 days.	Bone cement was mixed with the Optivac vacuum mix system (Schering-Plough). The PMMA was pressed in blocks (5x1x0.4 cm) and placed in 20 ml normal saline which was changed after every time period.	Fluorescence polarization immunoassay (Abbot TDx system, Abbot Laboratories, Chicago, USA).	Unknown.
Holm and Vejlsgaard 1976	Better antibacterial efficacy over 30 days than in this study.	CMW™ 1 was manually mixed with 1 g gentamicin under atmospheric pressure and pressed in rods (10x4x110 mm). The rods were set in human serum which was changed daily.	Inhibition zone assay with 0.2 ml serum on 6 mm paper disc.	Unknown.
Torrado et al. 2001	Antibiotic elution over 51 days.	Bone cement was mixed under atmospheric pressure and pressed in spherical specimens (8 g) which were placed in the dissolution apparatus USP 23 1 with 150 ml PBS (United States Pharmacopeia 1995). After each time period, 2ml were taken out for analysis and filtered with a Whatman filter (0.45 mm).	Fluorescent polarization immunoassay (Abbot TDx system, Abbott Laboratories, Chicago, USA).	Autonomous Government (Comunidad de Madrid) and the CYCIT grant.

Table 17 con.: Conclusion of previous studies of CMW™ 1 G.

Simplex® T				
Reference	**Conclusion**	**Production**	**Measurement**	**Funding**
Corresponding to the results:				
Klekamp et al. 1999	Simplex® elutes tobramycin over the whole tested time period of four days.	Simplex® P was manually mixed with 1.2 g tobramycin under atmospheric pressure and pressed in moulds which were placed in 1 ml PBS with change after every measurement.	Enzyme-linked immuno-sorbent assay (Abbott TDX, Abbott Park, Illinois, USA).	Unknown.
Moojen et al. 2008	Release over seven days.	PMMA was mixed under atmospheric pressure and pressed in hip spacers (9x125 mm stem size, 51 mm head diameter). The spacer was placed in 1 l PBS from which 250 µl were periodically taken out for analysis.	Fluorescent polarizing immunoassay (Abbott AxSym system, Abbott Diagnostics, Hoofddorp, The Netherlands).	Biomet (The Netherlands) and Stryker (Benelux).
Deviate from the results:				
DiCicco et al. 2003	Tobramycin elutes only over 12 days from Simplex®.	Simplex® P was manually mixed with 1 g tobramycin under atmospheric pressure and pressed in blocks (5 cm^3). These blocks were placed in 45 ml PBS from which 25 µl were taken out daily for analysis.	Standard spectrofluoro-meter (FP-750 Jasco, Japan).	Unknown.
Karek et al. 2017	Antibiotic release for six weeks with growth inhibition of *Staphylococcus aureus*.	Simplex® P was manually mixed with 1 g tobramycin under atmospheric pressure. 3.5 mm guidewires were coated with the PMMA and set in PBS. Aliquots were taken out after each time period for analysis.	Unknown.	Orthopaedic Research and Education Foundation and Exactech.

Table 18: Conclusion of previous trials with Simplex® T.

16 studies compared the PMMA of Palacos® with the bone cement of Simplex® (table 19) of which 13 came to corresponding results and described a better and longer elution or antibacterial efficacy of Palacos® [Elson et al. 1977; Greene et al. 1998; Hoff et al. 1981; Holm and Vejlsgaard 1976; Kendall et al. 1996; Klekamp et al. 1999; Kuechle et al. 1991; Kühn et al. 2016; Lee et al. 2016; Marks et al. 1976; Picknell et al. 1979; Stevens et al. 2005; Wahlig and Dingeldein 1980], while three references stated a superiority of Simplex® [Cerretani et al. 2002; Moojen et al. 2008; Picknell et al. 1979]. The comparison of CMW™ and Palacos® shows similar results. Table 20 shows nine studies which described a dominance of Palacos® [Armstrong et al. 2002; Bayston and Milner 1982; Elson et al. 1977; Holm and Vejlsgaard 1976; Kühn et al. 2016; Lee et al. 2016; Neut et al. 2003; Penner et al. 1999; Van De Belt et al. 2000], while three trials stated opposite results [Bayston and Milner 1982; Ensing et al. 2005; Hendriks et al. 2005]. Furthermore, table 21 shows five publications which compared Simplex® with CMW™ of which two described a higher elution or better antibacterial efficacy from Simplex® [Holm and Vejlsgaard 1976; Kühn et al. 2016], one stated an equal antibacterial efficacy [Elson et al. 1977] and two showed a dominance of CMW™ [Lee et al. 2016; Wahlig and Dingeldein 1980].

Palacos® versus Simplex®		
Reference	**Antibiotic**	**Conclusion**
Corresponding to the results:		
Elson et al. 1977	Gentamicin	Palacos® has a better and longer antibacterial efficacy than Simplex® in plate diffusion assay over 24 days. Palacos® is 200 percent more effective than Simplex®.
Greene et al. 1998	Tobramycin, vancomycin	Palacos® has a better and longer antibiotic release than Simplex®.
Hoff et al. 1981	Gentamicin, penicillin	Palacos® has a better elution and antibacterial efficacy than Simplex® in isotopic and agar diffusion assay over six weeks.
Holm and Vejlsgaard 1976	Gentamicin	Palacos® has a longer antibacterial efficacy (>90 d) than Simplex® (90 d), but Simplex® has greater inhibiting areolas than Palacos® in agar diffusion assay.
Kendall et al. 1996	Tobramycin, vancomycin	Palacos® has a better antibacterial efficacy than Simplex® in special agar diffusion assay.
Klekamp et al. 1999	Vancomycin	Palacos® has a minimal better elution than Simplex® in enzyme-linked immunosorbent assay.
Kuechle et al. 1991	Vancomycin, daptomycin, amikalin	Vancomycin and amikalin have a higher release from Palacos® than from Simplex®. The elution of daptomycin is equal in both cements.
Kühn et al. 2016	Palacos R+G® versus Simplex T®	Palacos® has a 0.8 times higher release than Simplex® after seven days.
Lee et al. 2016	Vancomycin	Palacos® is better in elution than Simplex® in fluorescence polarized immunoassay.
Marks et al. 1976	Gentamicin	Palacos® has a better and longer release than Simplex®.
Picknell et al. 1979	Gentamicin, carbenicillin	Palacos® has a better elution than Simplex® in agar diffusion assay over 24 hours (3.7% versus 7.1% by gentamicin).

Table 19: Comparison of bone cements from Palacos® and Simplex® in literature.

Stevens et al. 2005	Tobramycin, vancomycin	Palacos® has a better and longer antibacterial efficacy than Simplex® in agar diffusion assay.
Wahlig and Dingeldein 1980	Gentamicin	Palacos® has a 2.8 times higher release than Simplex® in agar diffusion test (286 mg versus 100 mg).
Deviate from the results:		
Cerretani et al. 2002	Vancomycin, vancomycin with imipenem-cilastatin	Simplex® has an equal elution than Palacos® in high-performance liquid chromatography analysis. Palacos® with vancomycin has a longer release time than Simplex®, it has a shorter elution period with vancomycin with imipenem-cilastatin.
Moojen et al. 2008	Palacos R+G® versus Simplex T®	Both cements have an equal release in fluorescence polarizing immunoassay over one week.
Picknell et al. 1979	Amoxycillin, cloxacillin, flucloxacillin	Simplex® has a better antibacterial efficacy than Palacos® in agar diffusion assay.

Table 19 con.: Comparison of bone cements from Palacos® and Simplex® in literature.

CMW™ 1 G versus Palacos®		
Reference	**Antibiotic**	**Conclusion**
Corresponding to the results:		
Armstrong et al. 2002	Gentamicin, vancomycin	Palacos® has a greater elution than CMW™ in fluorescence polarization immunoassay over 21 days.
Bayston and Milner 1982	Gentamicin	Palacos® has a longer antibacterial efficacy than CMW™ in agar diffusion assay with serial plate transfer (eight days versus 13 days).
Elson et al. 1977	Gentamicin	Palacos® has a better and longer antibacterial efficacy than CMW™ in agar diffusion assay over 24 days. Palacos® is 200 percent more effective than CMW™.

Table 20: Conclusion of studies which compare bone cements from CMW™ and Palacos®.

Holm and Vejlsgaard 1976	Gentamicin	Palacos® has a greater and longer antibacterial efficacy than CMW™ in inhibition zone assay (>90 days versus 31 days).
Kühn et al. 2016	Gentamicin	Palacos® has nine times more elution than CMW™ 1 G after seven days.
Lee et al. 2016	Vancomycin	Palacos® is better in antibiotic release than CMW™ in fluorescence polarized immunoassay.
Neut et al. 2003	Gentamicin	Palacos® has a higher elution than CMW™ 1 G in fluorescence polarized immunoassay (3.46-3.84% versus 1.32-1.56%).
Penner et al. 1999	Tobramycin, vancomycin	CMW™ has a 26 percent lower release than Palacos® with tobramycin and 36 percent less with vancomycin in fluorescence polarized immunoassay.
Van De Belt et al. 2000	Gentamicin	Palacos® has a greater elution than CMW™ in fluorescence polarization immunoassay (5.3% versus 8.4%)
Deviate from the results:		
Bayston and Milner 1982	Sodium fusidate, diethanolamine fusidate	CMW™ has a longer antibacterial efficacy than Palacos® with sodium fusidate (19 days versus 50 days) and diethanolamine fusidate (five days versus seven days) in agar diffusion assay with serial plate transfer.
Ensing et al. 2005	Gentamicin	CMW™ has a higher elution than Palacos® in fluorescence polarizing immunoassay after 18 hours (90 µg/cm^2 versus 55 µg/cm^2).
Hendriks et al. 2005	Gentamicin	CMW™ 1 G has a better release than Palacos® R+G in fluorescence polarized immunoassay.

Table 20 con.: Conclusion of studies which compare bone cements from Palacos® and Simplex®.

CMW™ 1 G versus Simplex®		
Reference	**Antibiotic**	**Conclusion**
Corresponding to the results:		
Holm and Vejlsgaard 1976	Gentamicin	Simplex® has a longer antibacterial efficacy than CMW™ in inhibition zone assay (90 days versus 31 days).
Kühn et al. 2016	CMW™ 1 G versus Simplex® T	Simplex® has a higher elution than CMW™ 1 G.
Deviate from the results:		
Elson et al. 1977	Gentamicin	CMW™ has the same antibacterial efficacy than Simplex® in standard plate diffusion assay.
Lee et al. 2016	Vancomycin	CMW™ has a better release than Simplex® in fluorescence polarized immunoassay.
Wahlig and Dingeldein 1980	Gentamicin	CMW™ has a higher antibiotic elution than Simplex® in agar diffusion assay (121 mg versus 100 mg).

Table 21: Comparison of CMW™ and Simplex® bone cement in literature.

Table 16, 17 and 18 show that four different methods were commonly used to analyse antibiotics in serum or solutions: agar diffusion assay, fluorescence polarizing immunoassay, liquid chromatography and isotopic assay. The agar diffusion assay is a qualitative test which shows the antibacterial efficacy of a solution against a certain bacterium. Exact quantitative analyses are not possible, but with the help of standard dilutions, a calibration curve can be created which allows approximate statements. The test precision is dependent on the minimum inhibitory concentration (MIC) of the bacteria. The higher the MIC the lower is the precision of the inhibition zones. Fluorescence polarizing immunoassay, liquid chromatography and isotopic assay are quantitative tests which show the exact proportion of antibiotic in a fluid. However, these assays also measure biologically inactive antibiotic molecules in addition to the biologically active ones. According to Hoff et al. [1981] this disadvantage can be neglected because of the minimal difference. They measured a difference between qualitative and quantitative tests of 0.7 percent in the tests with gentamicin and 0.05 percent with penicillin which are both in the standard error of the tests.

4.3.1 Simplex® HVG

Data to elution rates or antibacterial efficacy could not be found.

4.3.2 Hi-Fatigue® G

Gallo et al. [2013] compared the bone cements Palacos® R+G and Hi-Fatigue® G. Both PMMA eluted over at least eight days, whereby Hi-Fatigue® G reached a higher total elution then Palacos® R+G with 162.14 mg/l versus 83.36 mg/l in immunoassay analysis. The authors deviate therefore from the results of this study where Palacos® R+G reached the best antibacterial efficacy of all bone cements with one antibiotic.

4.3.3 BonOs® R Genta

No publications regarding the bone cement BonOs® R Genta could be found in PubMed. Osartis GmbH [2019], the manufacturer of the BonOs® cement, describes an antibiotic elution over at least 10 days. This is similar to the results were the PMMA showed an antibacterial efficacy up to 28 days.

4.3.4 Copal® G+C

Corresponding to the results of this study, Boelch et al. [2017], Ensing et al. [2008] and Roth et al. [2015] described an antibiotic elution or antibacterial efficacy over at least six weeks, four weeks and ten days, respectively. Roth et al. [2015] tested the PMMA with agar diffusion tests, whereby it inhibited MSSA, MRSA and *Bacillus subtilis* over the whole tested time period of ten days.

4.3.5 Refobacin® Revision

Data to elution rates or antibacterial efficacy could not be found.

4.3.6 Simplex® E+C

Ruzaimi et al. [2006] tested this bone cement with agar diffusion tests against seven different bacteria over five days. In conclusion, Simplex® E+C was effective against *Staphylococcus aureus*, coagulase-negative staphylococci, *Klebsiella species*,

Pseudomonas species and E. coli. Tests with *Enterococcus species* and *Proteus species* showed no zones of inhibition. The main antibacterial efficacy was described in the first 72 hours.

4.3.7 Vancogenx® and Copal® G+V

Next to Palacos® R+G and Hi-Fatigue® G, Gallo et al. [2013] furthermore tested the bone cement Vancogenx®. The PMMA eluted gentamicin and vancomycin over at least eight days. The total amount of gentamicin was measured with an immunoassay and reached 166.46 mg/l. The total release of vancomycin was 190.999 mg/l analysed with high pressure liquid chromatography. In contrast to this study, Vancogenx® showed a higher burst release on the first day with elutions of 73.2 percent of vancomycin and 78.9 percent of gentamicin in the first 24 hours.

Copal® was tested two times by Boelch et al. [2017, 2018]: on the one hand, the industrially mixed Copal® G+V and on the other hand, Copal® spacem with manual loading with the same antibiotics in the same concentrations (0.5 g gentamicin and 2.0 g vancomycin). Copal® eluted both times over the whole testing time period of six and four weeks, respectively. This corresponds to the results of this study.

Kendoff et al. [2016] tested the antibiotic elution of Copal® G+V in an in-vivo setting with 20 patients. Plasma levels of gentamicin and vancomycin were quantified over seven days with maximum concentrations of 209.65 ng/ml gentamicin and 134.64 ng/ml vancomycin, respectively.

The bacteria chosen for the tests allow a detailed interpretation of the antibacterial efficacy. E. coli is sensitive to gentamicin and resistant to vancomycin, while MRSA is resistant to gentamicin and sensitive to vancomycin. Therefore, it is possible to evaluate the antibacterial efficacy of the single antibiotics in the bone cements which contain aminoglycoside and glycopeptid.

Vancogenx® showed different zones of inhibition for the bacteria. This PMMA showed an antibacterial efficacy of gentamicin up to seven days and of vancomycin three times longer. Copal® G+V had the same antibacterial efficacy for both antibiotics except in the time period between day one and seven, where gentamicin reached a better effect.

4.4 Theories for differences in elution characteristics

The elution process of antibiotics out of polymethylmethacrylate and the reasons for the different elution rates and antibacterial efficacies of different brands are a well discussed theme in literature. Every brand uses a different mixture of contents for their PMMA which therefore leads to different bone cements with different elution characteristics [Elson et al. 1977; Kühn et al. 2016]. When the PMMA is manually blended, the antibiotic and its byproducts in the powder also influence the cement structure, because of different chemical interactions with the PMMA [Lee et al. 2016]. Many authors described the theory that the high initial elution of antibiotics out of PMMA is caused by diffusion from the surface which is the higher the larger the surface [Ensing et al. 2005; Lee et al. 2016; Miola et al. 2013; Neut et al. 2003; Van De Belt et al. 2000]. While many authors explained that the total amount of the elution depends on the bulk porosity, or more specifically the size and the numbers of the pores in the bone cement [Hendriks et al. 2005; Lee et al. 2016; Miola et al. 2013; Neut et al. 2003; Van De Belt et al. 2000], others had the theory that the total release is caused by the diffusion through a permeable matrix, because porosimetry studies could not confirm the other theory [Elson et al. 1977; Ensing et al. 2005].

4.5 Limitations

The in vitro design of this study is a big limitation. The polymethylmethacrylates were tested under completely different conditions than the state in the human body is. The effect of mechanical load and friction were not considers as well as the fluid circulation which is influenced by the bone and soft tissue which produces and resorbs the synovial fluid continuously. Furthermore, drainages biased the local antibiotic concentration by draining the wound secret out of the body.

In addition, the tests took place by room temperature and not by 37 degrees Celsius which can also affect the drug elution.

In conclusion, the in vivo conditions cannot be imitated in laboratory because many (unknown) factors affect the conditions in the human body. It can be expected that the differences in antibacterial efficacy of the respective bone cements also exist in the human body, but that is not guaranteed.

5 Conclusion

A precise germ determination and the choice of the right drug-loaded cement with a good antibacterial efficacy over a long time is essential to prevent unsuccessful treatments of local infections after total joint replacements and thereby an important loss of time and damage to the joint. In total, only four cements showed a relevant antibacterial efficacy over 42 days: Palacos® R+G, Refobacin® Revision, Copal® G+C and Copal® G+V.

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7 Attachment

7.1 Project schedule

FB-OSL-04(06)
Konzeptformular für wissenschaftliche Arbeiten

Medizinische Universität Graz

(Arbeits-)Titel *Das Thema der Diplomarbeit ist einem der im Curriculum festgelegten Prüfungsfächer zu entnehmen.*	Comparative analysis with drug-loaded cements for the usage in revision arthroplasty
Untertitel (optional)	
Konzept erstellt von: Erstellt am: Revisionsdatum bei Änderungen: Version: *(Erste eingereichte Version ist „01“)* Matrikelnummer: Studienkennzahl:	Julian Kunzi 08.03.2017 01 1333245 O202
Betreuer/in: Institut/Klinik: Kontakt: *(Adresse, Telefonnummer, E-Mail)* Zweite/r Betreuer/in: Kontakt: *(Adresse, Telefonnummer, E-Mail)* MitarbeiterInnen:	Prof. Dr. rer. nat. Klaus-Dieter Kühn Universitätsklinik für Orthopädie und Traumatologie Auenbruggerplatz 5, 8036 Graz; +4915114824959; klaus.kühn@medunigraz.at Univ.-Prof. Dr. med. univ. Andreas Leithner Auenbruggerplatz 5, 8036 Graz; +4331638514807; andreas.leithner@medunigraz.at PD Dr. med. Andrej Trampuz; Magdalena Czuban, MSc; Mariagrazia Di Luca, PhD (Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Mittelallee 4, 13353 Berlin)
Kernfrage und Zielsetzung *Wie lautet die Fragestellung?* *Warum ist diese Frage von Bedeutung?* *Welche Ergebnisse sind im Wesentlichen zu erwarten?* *Worin besteht der theoretische Kern der Arbeit?* Sind die Forschungsfrage und die mit dem Projekt angestrebte Zielsetzung für Männer und Frauen gleichermaßen bedeutsam? Detaillierte Angaben!	Auf dem Markt befinden sich viele verschiedene antibiotikaversetzte PMMA-Zemente für den Einsatz in Revisionsarthroplastien. Es gibt Case Reports, bei denen Zementspacer trotz Einsatz dieser Zemente von Bakterien kolonisiert wurden und somit die kombinierte operative und medikamentöse Therapie fehlgeschlagen ist. Die zentrale Frage ist, wie das Antibiotikaabgabeverhalten der verschiedenen Zemente ist. Dadurch sollen Zemente enttarnt werden, die keine ausreichende Antibiotikakonzentration erreichen und somit keine lokale Therapie ermöglichen. Es ist zu erwarten, dass Die Forschungsfrage ist für Männer und Frauen gleichbedeutsam.
Kurzbeschreibung (max. 20 Zeilen) *Worin besteht der Neuigkeitswert?*	Auf dem Markt sind viele verschiedene antibiotikabeladene Zemente verfügbar. Diese Studie soll zeigen, dass einige der Zemente keine so optimale Antibiotikafreisetzung haben, wie andere. Dadurch soll die Grundlage für eine wissenschaftlich fundierte Empfehlungsbroschüre gelegt werden.
Methodenwahl *Welche Methoden stehen zur Beantwortung der Frage zur Verfügung? Wieso wählen Sie genau diese Methode?*	Die Zementformkörper werden in Phosphatpuffer in verschiedenen Zeitabständen eingelegt, sodass das Antibiotikum unter körperähnlichen Situationen freigesetzt wird. Anschließend wird das Eluat auf Agarplatten gegeben, die mit verschiedenen Keimen beimpft wurden. Anhand der Hemmhöfe wird die Freisetzung verglichen. Diese Methode wurde gewählt, da sie neben der Flüssigchromatographie mit Massenspektrometrie-Kopplung die gängige Methode in der Literatur ist und somit sehr gut mit anderen Studien vergleichbar ist.

Ethikkommissionsvotum *Ist ein Votum der Ethikkommission erforderlich?* *Siehe Informationsblatt „Genehmigung Ethikkommission“*	☐ Erforderlich ☒ Nicht erforderlich ☐ Bereits vorhanden
Datenerhebung (falls zutreffend) *Werden aufgrund der oben genannten Methodenwahl medizinische Daten benötigt? Wenn ja, welche?* *Mit welcher Fallzahl ist zu rechnen? Wie wurde die Fallzahl ermittelt?* *Wie ist das PatientInnenkollektiv zu beschreiben (Mindest-/Höchstalter, Geschlechtsverteilung, Begleiterkrankungen, etc.)?* *Bitte beachten Sie, dass eine Weitergabe der Daten an projektfremde Personen gemäß Datenschutzgesetz nicht zulässig ist. Das Bekanntwerden von PatientInnendaten ist durch Pseudonymisierung (Codierung mit fortlaufender Nummer) und ggf. Zugriffsbeschränkungen zu verhindern.*	Nicht zutreffend
Datenauswertung *Welche Hauptzielgröße wird analysiert (z.B. Alter bei Diagnosestellung/Alter bei Operation/Diagnose, etc.)? Wie wird die Hauptzielgröße analysiert?* *Welche Nebenzielparameter sollen betrachtet werden? Mit welchen Methoden erfolgt die Auswertung?*	Die Hauptzielgröße ist der Komplettdurchmesser der Hemmhöfe auf den Agarplatten, der mit einem Lineal gemessen wird. Diese Messwerte werden mittels statistischer Verfahren auf signifikante Unterschiede untersucht.
Zeitplan (grob strukturiert) *Wann wird mit der Arbeit begonnen? Wann wird ein Antrag bei der Ethikkommission gestellt, sofern ein Votum erforderlich ist? Welche Meilensteine wurden zwischen dem/der Studierenden und den BetreuerInnen vereinbart? Wann ist voraussichtlich mit der Beendigung der Arbeit zu rechnen?* *Welche formalen Schritte sind für die Umsetzung der Diplomarbeit notwendig?*	05.02.2017: Start der Laborarbeit an der Charité in Berlin mit Arbeitskonzeptentwicklung und Durchführung 10.04.2017: voraussichtliches Ende der Laborarbeit 01.10.2017: voraussichtliches Ende der Diplomarbeit
Referenzen Welche Literatur ist relevant? Gibt es Vergleichsstudien?	Eine erste Recherche zeigt folgende relevante Literatur: CORACA-HUBER, D., HAUSDORFER, J., FILLE, M., NOGLER, M. and KUHN, K.D., 2014. Calcium carbonate powder containing gentamicin for mixing with bone grafts. *Orthopedics,* **37**(8), pp. e669-72. CORACA-HUBER, D.C., PUTZER, D., FILLE, M., HAUSDORFER, J., NOGLER, M. and KUHN, K.D., 2014. Gentamicin palmitate as a new antibiotic formulation for mixing with bone tissue and local release. *Cell and Tissue Banking,* **15**(1), pp. 139-144. HSU, Y.H., HU, C.C., HSIEH, P.H., SHIH, H.N., UENG, S.W. and CHANG, Y., 2017. Vancomycin and Ceftazidime in Bone Cement as a Potentially Effective Treatment for Knee Periprosthetic Joint Infection. *The Journal of bone and joint surgery.American volume,* **99**(3), pp. 223-231. KIENAPFEL, H. and KÜHN, KD., eds, 2009. *The Infected Implant.* Heidelberg, Germany: Springer Medizin Verlag. WALENKAMP, G., ed, 2007. *Local Antibiotics in Arthroplasty - State of the Art from an interdisciplinary point of view.* Stuttgart, Germany: Georg Thieme Verlag. YUENYONGVIWAT, V., INGVIYA, N., PATHABUREE, P. and TANGTRAKULWANICH, B., 2017. Inhibitory effects of vancomycin and fosfomycin on methicillin-resistant Staphylococcus aureus from antibiotic-impregnated articulating cement spacers. *Bone & joint research,* **6**(3), pp. 132-136.

Benötigte Ressourcen *Werden Geld- oder Sachmittel von Einrichtungen der Med Uni Graz benötigt?* Die Vergabe ist nur zulässig, wenn die Leiterin/der Leiter dieser Einrichtung über die beabsichtigte Vergabe informiert wurde und diese nicht binnen eines Monats untersagt hat.	Da die Laborversuche an der Charité in Berlin durchgeführt werden, fallen nur die Reisekosten nach Berlin an. Hierfür wird probiert ein Förderungs-stipendium der Medizinischen Universität Graz zu bekommen. Anderenfalls übernimmt die Abteilung für Orthopädie und Traumatologie des LKH Graz die Kosten.

7.2 Microbial sensitivity tests with MRSA strain ATCC 43300

Labor Berlin 1

bioMerieux-Kunde: 1040571
Systemnr.: 3632 3627

Laborbefund

Gedruckt am 08.05.2015 14:16 CEST
Gedruckt von: labsuper

*** Angewandter Alarm ***

Referenznummer: NWV14697-1

Bionummer: 050402062763031
Gewählter Keim: Staphylococcus aureus (MRSA ATCC 43300)

Infos zur Resistenz	Karte:	AST-P632	Chargenbez:	732347210	Verfallsdatum:	16.06.2016 13:00 CEST
	Beendet:	08.05.2015 00:30 CEST	Status:	Fertig	Analysen-Dauer:	10,00 Std.

Antibiotikum	MHK	Interpretation	Antibiotikum	MHK	Interpretation
Cefoxitin-Screen	POS	+	Levofloxacin	0,25	S
Benzylpenicillin	>= 0,5	R	+Moxifloxacin		S
+Ampicillin		R	Induzierbare Clindamycin Resistenz	NEG	-
+Ampicillin/Sulbactam		R	Erythromycin	>= 8	R
+Piperacillin		R	Clindamycin	>= 4	R
+Piperacillin/Tazobactam		R	Linezolid	2	S
Oxacillin	>= 4	R	Daptomycin	0,5	S
+Cefazolin		R	Teicoplanin	<= 0,5	S
+Cefuroxim		R	Vancomycin	1	S
+Ceftazidim		R	Tetracyclin	<= 1	S
+Ceftriaxon		R	Tigecycline	<= 0,12	S
+Imipenem		R	Fosfomycin	<= 8	S
+Meropenem		R	Fusidinsäure	<= 0,5	S
Gentamicin	>= 16	R	Mupirocin	<= 2	S
+Tobramycin		R	Rifampicin	<= 0,03	S
+Ciprofloxacin		S	Trimethoprim/Sulfamethoxazol	<= 10	S

+= Abgeleitete Antibiotika *= AES modifiziert **= Anwender modifiziert

AES-Befunde:		Letzte Änderung: 05.08.2014 11:04 CEST	Parameterset:	EUCAST/CASFM + PHAENOTYPISCH 2014 D
Zuverlässigkeit-Ebene:	Konsistent			
Phänotyp:	BETA-LAKTAM-ANTIBIOTIKA	PBP MODIFIKATION (mecA)		

Aktion	Name (Anwender ID)	Datum/Uhrzeit	Kommentar
Geprüft von:	(labsuper)	08.05.2015 09:05 CEST	

Installierte VITEK 2 Systems Version: 06.01
MHK-Interpretationsrichtlinie: EUCAST + CASFM 2014 D

Therapeutische Interpretationsrichtlinie: DEUTSCHLAND PHENOTYPIC 2014

Bezeichnung des AES-Parametersets: EUCAST/CASFM + PHAENOTYPISCH 2014 D

Letzte Änderung der AES-Parameter: 05.08.2014 11:04 CEST

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