Materials. Clin Orthop Relat Res. 2016;474:1649–1656. doi:10.1007/s11999-016-4720-8.
[10] Antunes ALS, Bonfanti JW, Perez LRR, Pinto CCF, Freitas ALP de, Macedo AJ, et al. High vancomycin resistance among biofilms produced by Staphylococcus species isolated from central venous catheters. Mem Inst Oswaldo Cruz. 2011;106:51–55.
[11] Antunes ALS, Trentin DS, Bonfanti JW, Pinto CCF, Perez LRR, Macedo AJ, et al. Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. APMIS. 2010;118:873–877. doi:10.1111/j.1600-0463.2010.02681.x.
[12] Ray C, Shenoy AT, Orihuela CJ, González-Juarbe N. Killing of Serratia marcescens biofilms with chloramphenicol. Ann Clin Microbiol Antimicrob. 2017;16:19. doi:10.1186/s12941-017-0192-2.
[13] Reiter KC, Sambrano GE, Villa B, Paim TG da S, de Oliveira CF, d'Azevedo PA. Rifampicin fails to eradicate mature biofilm formed by methicillin-resistant Staphylococcus aureus. Rev Soc Bras Med Trop. 2012;45:471–474.
[14] Ruppen C, Hemphill A, Sendi P. In vitro activity of gentamicin as an adjunct to penicillin against biofilm group B Streptococcus. J Antimicrob Chemother. 2017;72:444–447. doi:10.1093/jac/dkw447.
[15] Hajdu S, Lassnigg A, Graninger W, Hirschl AM, Presterl E. Effects of vancomycin, daptomycin, fosfomycin, tigecycline, and ceftriaxone on Staphylococcus epidermidis biofilms. J Orthop Res. 2009;27:1361–1365. doi:10.1002/jor.20902.
[16] Ravn C, Furustrand Tafin U, Bétrisey B, Overgaard S, Trampuz A. Reduced ability to detect surface-related biofilm bacteria after antibiotic exposure under in vitro conditions. Acta Orthop. 2016;87:644–650. doi:10.1080/17453674.2016.1246795.
[17] Monzón M, Oteiza C, Leiva J, Lamata M, Amorena B. Biofilm testing of Staphylococcus epidermidis clinical isolates: low performance of vancomycin in relation to other antibiotics. Diagn Microbiol Infect Dis. 2002;44:319–324.
[18] Molina-Manso D, del Prado G, Ortiz-Pérez A, Manrubia-Cobo M, Gómez-Barrena E, Cordero-Ampuero J, et al. In vitro susceptibility to antibiotics of staphylococci in biofilms isolated from orthopaedic infections. Int J Antimicrob Agents. 2013;41:521–523. doi:10.1016/j.ijantimicag.2013.02.018.
[19] Claessens J, Roriz M, Merckx R, Baatsen P, Van Mellaert L, Van Eldere J. Inefficacy of vancomycin and teicoplanin in eradicating and killing Staphylococcus epidermidis biofilms in vitro. Int J Antimicrob Agents. 2015;45:368–375. doi:10.1016/j.ijantimicag.2014.11.011.
[20] Castaneda P, McLaren A, Tavaziva G, Overstreet D. Biofilm antimicrobial susceptibility increases with antimicrobial exposure time. Clin Orthop Relat Res. 2016;474:1659–1664. doi:10.1007/s11999-016-4700-z.

Authors: Jan Geurts, Williem Berend Schreurs, Jean-Yves Jenny

QUESTION 4: What is the minimum biofilm eradication concentration (MBEC) of anti-infective agents?

RESPONSE/RECOMMENDATION: The MBEC of antimicrobial agents is a measure of in vitro antibiotic susceptibility of biofilm producing infective organisms. It is dependent on the surface, medium and the exposure period to an antimicrobial agent. There are no standardized measurement parameters for MBEC. MBEC is currently a research laboratory value and lacks clinical availability. In the group's opinion, there is value in developing a clinically-validated MBEC assay.

LEVEL OF EVIDENCE: Consensus

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

PRE-MEETING RATIONALE

A Medline query on the item "minimum biofilm eradication concentration" retrieved 149 references. For the most part, these references relate to bacteria with little or no involvement in infection on orthopaedic devices. A query about "minimum biofilm eradication concentration of infective agents" retrieved 18 references; none of them clearly related to bone infection on material. The Medline request "minimum biofilm eradication concentration and implant associated infection" retrieves only three references [1–3].

The work of Coraça-Huber et al. [1] focuses on the evaluation of a study model of the minimum bacterial concentration (MBC) in infections on material, using strains of *Staphylococcus aureus (S. aureus)* and collection *Staphylococcus epidermidis (S. epidermids)*. Biofilm formation is supported by Innovotech, Inc.'s MBEC-HTP (high throughput plates) system (Edmonton, Alberta, Canada). The formation of biofilm is documented by electron microscopic study. The comparison of the minimum inhibitory concentration (MIC) and MBEC was made in this model for daptomycin, gentamicin, vancomycin, rifampicin, fosfomycin, clindamycin and linezolide. Biofilms generated by *S. epidermidis* show less resistance to antibiotics than those generated by *S. aureus*. The MBEC is much higher than the MIC of all antibiotics. Daptomycin and rifampicin are the most effective antibiotics against *S. aureus* embedded within a biofilm without obtaining their complete eradication.

Brady et al. [2] raised a question about the validity of the MBEC to replace the IJC in situations of infection on equipment. Twenty staphylococcal isolates from catheter infections were studied (17 CNS, 3 MSSA) and ten antibiotics were tested (penicillin, oxacillin, erythromycin, clindamycin, fucidine, tetracycline, gentamicin, vancomycin, teicoplanin and ciprofloxacin). The quantification of biofilm formation on microtiter plates and Tryptic Soy Broth (TSB) is obtained by crystal violet method. Detection of the biofilm formation mechanism (protein or polysaccharide) is obtained by treatment of sodium metaperiodate and protein kinase plates. The search for the ica operon (code in staphylococci for the production of enzymes necessary for adhesion) is done by polymerase chain reaction. Sixteen of the 20 strains (80%) tested produce biofilm; low for 8 strains, moderate for 2 strains, and high for 6 strains, all carriers of ica operons. The MBEC was 10 to 1,000 times higher than the MIC for bacteria producing biofilm. In practice, the MBC is > 256 µg/ml for all strains studied, whether or not biofilm production is proven by the techniques used, raising the question of strains forming a protein biofilm that cannot be quantified by the crystal violet method.

Zaborowska et al. [3] analyzed the sensitivity of staphylococci and enterococci from bone infections on material according to their biofilm production. The 13 strains studied were derived from infections on percutaneous bone anchoring material, on femoral amputation stumps for fitting. This technique involves a permanent protrusion of a titanium implant through the skin, a potential entry point for bacteria from the cutaneous and fecal flora. The bacteria studied were obtained from bone and material samples obtained from 11 infected patients. These are four strains of *S. aureus*, three strains of coagulase-negative staphylococci and six strains of *Enterococcus faecalis*. Ten antibiotics are tested in MIC and MBEC (clindamycin, gentamicin, vancomycin, linezolide, ciprofloxacin, oxacillin, fucidic acid, ampicillin, trimethoprim/sulfamethoxazole and rifampicin). The microtiter plate culture in TSB is used to evaluate the biofilm

production capacity of the bacteria analyzed. The total mass of the biofilm formed is measured by the crystal violet technique to determine a biofilm score (absent, low, moderate, high production). The production of exopolysaccharide (slime) is measured by the Congo red technique. The search for the ica operon for staphylococci is obtained by PCR test. The determination of the MBEC is obtained by the Calgary Biofilm Device (CBD). Eleven of the 13 strains studied produce biofilm, the quantity of biofilm is heterogeneous according to bacterial species. The MBEC is significantly higher than the MIC for the 10 antibiotics studied. The ratio MBEC/MIC is variable with marked differences between bacterial species. The MBEC is high and homogeneous for all strains of *Enterococcus faecalis*: MBEC/MIC from 64 to 2048, median 512, for vancomycin, ciprofloxacin, linezolide, ampicillin and rifampicin. In comparison, Staphylococcus strains show significant inter strain variability; for *S. aureus* MBEC/MIC ranges from 1 to 2048, median to 9, for the 10 antibiotics tested. For *S. epidermidis* the ratio ranges from 0.0038 to 64, median to 1. The ica operon is isolated for all staphylococci; however, two strains do not produce slime by referring to the Congo red technique, expressing variability in gene expression. For these two strains, the biofilm score assessed by the crystal violet method was strongly positive, indicating that this biofilm consisted mainly of aggregated cells without slime production.

The clinical follow-up of the 11 patients was correlated to the results expressed in MBEC. Failure was correlated with a high MBEC value without statistical evidence. Two patients did not present any complications (recurrence, reinfection or need for material removal). For one, the strain did not produce biofilm; for the other, biofilm production was low. For other strains with low to moderate biofilm production, patients experienced one or two complications. One patient developed all three complications and the infecting strain was highly biofilm producing.

Of these three studies, only Zaborowska et al.'s [3] corresponds to a clinical situation of infection on an orthopaedic device. As in the other two studies, the work presented here only tests antibiotics as monotherapy, whereas clinical use is readily with dual therapy, particularly when rifampicin is prescribed. The work of Saginur et al. [4] on 17 strains of *S. epidermidis*, 11 strains of methicillin-susceptible *Staphylococcus aureus* (MSSA) and 12 strains of methicillin-resistant *Staphylococcus aureus* (MRSA), isolated from infections on material tested in MIC and MBEC (CBD device) 9 antibiotics in monotherapy and 94 combinations of antibiotics in bi or tritherapy. The MBEC is significantly higher than the MIC, but a significant heterogeneity between strains is also found in monotherapy. Among the 94 antibiotic combinations tested, 11 are bactericidal on more than 90% of MSSA strains growing in biofilm and 9 are for *S. epidermidis*. Rifampicin is the antibiotic most often present in these combinations.

The efficacy of antibiotics against bacteria growing in a biofilm, is generally explored in vitro under standardized, brief conditions of exposure of the bacterial strain to the antibiotic tested. In clinical practice, exposure to antibiotics is prolonged [5]. In this work, bacterial strains (MSSA, MRSA, *S. epidermidis*, *E. coli*, *Pseudomonas aeruginosa*) are tested for growth in a biofilm at varying antibiotic concentrations for three antibiotic exposure durations of one, three and five days. For most strains and antibiotics tested, the MBEC is significantly lower after 5 days of exposure to antibiotics than that measured after 24 hours of exposure.

It is commonly accepted that bacterial adhesion and bacterial growth within a biofilm, are the determinants of infection on material. It is also commonly accepted that the effectiveness of antibiotics within a biofilm is greatly diminished. Measurement of in vitro antibiotic activity by the MIC determined on planktonic bacteria is not predictive of in vivo antibiotic activity on bacteria growing in a biofilm. The MBEC is the supposedly most appropriate parameter for predicting the efficacy of antibiotics in vivo. The literature review shows that this parameter is over the last few years increasingly studied and taken into account to test antibiotics or various molecules against multiple microorganisms.

While the in vitro MBEC determination method itself is not problematic, the measurement of biofilm production is more random. Biofilm is made up of both bacterial cells and a substance of either a polysaccharide (slime) or protein nature. Not all bacteria produce biofilm. For staphylococci, the production of biofilm is linked to the existence of an operon (ica), detectable by PCR but whose expression is variable, and the highlighting of the operon does not mean slime production. The measurement of the overall mass of biofilm, generally by the crystal violet technique, which potentially defines biofilm scores (absent, weak, moderate, strong), does not necessarily account for the composition of this biofilm, likely to modify the MBC of antibiotics.

The capacity to produce biofilm is heterogeneous depending on the bacterial species. On the available data, the capacity to produce biofilm is strong for *Enterococcus faecalis* without inter-strain variability. For staphylococci, the capacity to produce biofilm seems more marked in *Staphylococcus aureus* than in staphylococcus epidermidis, but inter-strain variability is important for staphylococci. Rifampicin appears to be a more active antibiotic in biofilm than average. However, the rule is by no means absolute. The efficacy of antibiotic combinations is significantly superior to that of monotherapy molecules.

In a clinical situation, for a given strain, the MBEC cannot be estimated a priori, at least for staphylococci. Of the few published data, the MBEC still appears to be higher than at least 64 times the MIC for antibiotics active against *Enterococcus fecalis* (ampicillin, vancomycin, linezolide, rifampicin). For other bacteria, the MBEC of active antibiotics is not known.

There is no antibiotic combination that guarantees bacterial eradication in the biofilm for a given strain of staphylococcus, although antibiotic combinations are generally more effective than monotherapy treatments. The in vitro measurement of the MBEC is not a routine use for the moment. The research field needs to define a standardized methodology for possible use in clinical practice. High biofilm production appears to correlate with a higher complication or failure rate than low or absent biofilm production without statistical demonstration at this time.

REFERENCES

[1] Coraça-Hubér DC, Fille M, Hausdorfer J, Pfaller K, Nogler M. Evaluation of MBEC™ HTP biofilm model for studies of implant associated infections. J Orthop Research. 2012;30:1176–1180. doi:10.1002/jor.22065.

[2] Brady AJ, Laverty G, Gilpin DF, Kearney P, Tunney M. Antibiotic susceptibility of planktonic- and biofilm-grown staphylococci isolated from implant-associated infections: should MBEC and nature of biofilm formation replace MIC? J Med Microbiol. 2017;66:461–469. doi:10.1099/jmm.0.000466.

[3] Zaborowska M, Tillander J, Brånemark R, Hagberg L, Thomsen P, Trobos M. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants. J Biomed Mater Res B Appl Biomater. 2017;105:2630–2640. doi:10.1002/jbm.b.33803.

[4] Saginur R, Stdenis M, Ferris W, Aaron SD, Chan F, Lee C, et al. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob Agents Chemother. 2006;50:55–61. doi:10.1128/AAC.50.1.55-61.2006.

[5] Castaneda P, McLaren A, Tavaziva G, Overstreet D. Biofilm antimicrobial susceptibility increases with antimicrobial exposure time. Clin Orthop Relat Res. 2016;474:1659–1664. doi:10.1007/s11999-016-4700-z.