QUESTION 12: What is the Minimum Biofilm Eradication Concentration (MBEC) of anti-infective agents?

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Response/Recommendation:

The minimum biofilm eradication concentration (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)

Post Meeting Rationale:

A Medline query on "Minimum biofilm eradication concentration" retrieved 149 references. Majority of these were related to bacteria with little or no involvement in infection on orthopedic devices. A query about "Minimum biofilm eradication concentration of infective agents" retrieved 18 references, none of them directly related to bone infection on implants. A Medline search on "Minimum biofilm eradication concentration and implant associated infection" retrieved only three references [1–3].

Coraça-Huber et al. evaluated strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* in a biofilm model. Biofilm formation in the model was supported by Innovotech, Inc.'s MBEC-HTP (high throughput plates) system (Edmonton, Alberta, Canada) and the formation of biofilm was documented by electron microscopic study. The comparison of the MIC and MBEC was made in this model for daptomycin, gentamicin, vancomycin, rifampicin, fosfomycin, clindamycin and linezolid. Biofilms generated by *S. epidermidis* showed less resistance to antibiotics than those of *S. aureus*. The MBEC was much higher than the MIC for studied antibiotics. Daptomycin and rifampicin were the most effective antibiotics against *S. aureus* embedded within a biofilm , but they didn't achieve complete eradication[1].

Brady et al. raised a question about the validity of the MBEC to replace the MIC implant infections. Twenty staphylococcal isolates from catheter infections were studied (17 CNS, 3 MSSA) against penicillin, oxacillin, erythromycin, clindamycin, fucidine, tetracycline, gentamicin, vancomycin, teicoplanin and ciprofloxacin. Biofilm formation on microtiter plates and TBS broth was quantified by crystal violet method. Biofilm formation mechanism (protein or polysaccharide) was 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) was done by PCR. 16 of the 20 strains tested (80%) produced biofilm, low for 8 strains, moderate for 2 strains, and high for 6 strains, all carried of ica operons. The MECB was 10 to 1000 times higher than the MIC for bacteria producing biofilm[2].

Zaborowska et al. studied 4 strains of *Staphylococcus aureus*, 3 strains of coagulase-negative staphylococci and 6 strains of *Enterococcus faecalis* obtained from bone and material samples obtained among 11 infected patients. MIC and MBEC for clindamycin, gentamicin, vancomycin, linezolide, ciprofloxacin, oxacillin, fucidic acid, ampicillin, trimethoprim/sulfamethoxazole and rifampicin were tested. Microtiter plate culture in TBS broth was used to evaluate the biofilm production capacity of the bacteria analyzed. The total mass of the biofilm formed was measured by the crystal violet technique and a biofilm score (absent, low, moderate, high production) was noted. The production of exopolysaccharide (slime) was measured by the Congo red technique. The search for the ica operon for staphylococci was obtained by PCR. The determination of the MBEC was obtained by the CBD (Calgary Biofilm Device). 11 of the 13 strains studied produced biofilm, the quantity of biofilm was heterogeneous according to the species. The MBEC was significantly higher than the MIC for all antibiotics. The ratio MBEC/MIC was variable with marked differences between bacterial species. The MBEC was high and homogeneous for all strains of *Enterococcus faecalis*: MBEC/MIC ranged from 64 to 2048 (median 512), for vancomycin, ciprofloxacin, linezolid, ampicillin and rifampicin. In comparison, Staphylococcus strains show significant inter strain variability; For *Staphylococcus aureus* MBEC/MIC ranged from 1 to 2048 (median 9). For *Staphylococcus epidermidis* the ratio ranged from 0.0038 to 64 (median to 1). The ica operon is isolated for all staphylococci, however two strains didn't show slime by the congo red technique, but their biofilm score assessed by the crystal violet method was strongly positive, indicating that their biofilms consisted mainly of aggregated cells, without slime production [3].

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, re-infection or need for material removal). For one case, 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.

The work presented in these studies only tested antibiotics as monotherapy, whereas in clinical practice dual therapy, particularly combining with rifampicin to another agent in Staphylococcal infection is a common practice.

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 [4]. 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 and the capacity to produce biofilm is heterogeneous depending on the bacterial species.

The *in vitro* measurement of the MBEC is not a routine use for the moment, remains of the research field with the need to define a standardized methodology for possible use in clinical practice.

References:

1. Coraça-Hubér DC, Fille M, Hausdorfer J, et al. 2012. Evaluation of MBEC^{TM}-HTP biofilm model for studies of implant associated infections. J. Orthop. Res. 30(7):1176–1180.
2. Brady AJ, Laverty G, Gilpin DF, et al. 2017. 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. 66(4):461–469.
3. Zaborowska M, Tillander J, Brånemark R, et al. 2017. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants: BIOFILM FORMATION AND ANTIMICROBIAL SUSCEPTIBILITY. J. Biomed. Mater. Res. Part B Appl. Biomater. 105(8):2630–2640.
4. Castaneda P, McLaren A, Tavaziva G, Overstreet D. 2016. Biofilm Antimicrobial Susceptibility Increases With Antimicrobial Exposure Time. Clin. Orthop. Relat. Res. 474(7):1659–1664.