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QUESTION 3: What is the relevance of minimum inhibitory concentration (MIC) of infecting organisms in biofilm-mediated chronic infection?

RESPONSE: The use of MIC is limited to (1) defining antibiotics that the microorganism is susceptible to in its planktonic state but cannot be used to guide treatment of biofilm-based bacteria and (2) selecting long-term suppressive antibiotic regimens where eradication of infection is not anticipated. Alternative measures of antibiotic efficacy specifically in the context of biofilm-associated infection should be developed and validated.

LEVEL OF EVIDENCE: Strong

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

PRE-MEETING RATIONALE

MICs are used to define an individual microorganism's (hereafter limited to bacteria) susceptibility to a distinct array of antibiotics. Established methodologies for determining MICs relate to the planktonic state of the bacteria but not to biofilm-indwelling bacteria [1].

The majority of information relating to susceptibility testing and biofilm-indwelling bacteria originates from research in Cystic Fibrosis [2]. In relation to implant-associated biofilm infections, central venous catheters and urinary tract catheters are often investigated, but little clinical research has been performed in orthopaedic implant-associated biofilm infections [2,3].

As early as 1990, Anwar and Costerton identified the need for an extreme increase in in vitro concentrations of antibiotics, to which the planktonic bacteria were fully susceptible, when treating biofilm-indwelling bacteria [4,5]. In a review by key-opinion leaders on the topic of antimicrobial susceptibility testing in biofilm-indwelling

bacteria, it was noted that MIC is not suitable in predicting the effect of an antibiotic for a biofilm infection [6]. In the 2014 European Society for Clinical Microbiology and Infectious Diseases guidelines for the diagnosis and treatment of biofilms infections, it is noted that antibiotic susceptibility determination by MIC offers no guide to clinicians in the treatment of biofilms [7]. Rather than MICs, clinicians may need to rely on other measures of antibiotic efficacy, such as minimum biofilm eradication concentration (MBEC), minimum biofilm bactericidal concentration (MBBC) or minimum biofilm inhibitory concentration (MBIC). These are likely to be 100-1000 times the MIC, but the associated breakpoints that would permit reliable prediction of treatment success have not yet been established.

Theoretical mechanisms driving the high-level of resistance to antibiotics in biofilm include both the mechanical exclusion of antibiotic molecules by the polysaccharide matrix and the presence of dormant persister organisms within the biofilm. The relative contribution of each of these mechanisms is uncertain, but emerging data suggest that persister organisms constitute up to 10% of biofilm. Due to the adapted phenotype, they are able to evade the antimicrobial action of a variety of conventional antibiotics that rely on disruption of cell processes for their efficacy. Post et al. showed that, although it was possible to eradicate biofilm caused by *Staphylococcus aureus* (*S. aureus*), the necessary time-concentration profile could not be achieved in vivo by systemic administration or by any local delivery vehicles currently available [8]. Urish et al. concluded that tolerance was primarily a phenotypic phenomenon as increasing cefazolin exposure did not result in changes in MIC [9].

In two studies, Antunes et al. identified that among biofilm-indwelling Staphylococcus species isolates, 89% were considered to be clinical resistant to vancomycin, even when the same isolates all presented MIC values categorizing the isolates as fully susceptible to vancomycin (MIC </= 2µg/mL) [10,11]. The authors concluded that this particular observation showed "that biofilm production results in an important barrier to antimicrobial diffusion into the biofilm" and that "antimicrobial susceptibility testing based on MIC values alone cannot accurately determine the exact susceptibility of bacterial biofilms."

Ray et al. tested ceftriaxone and gentamicin, both commonly used antibiotics in orthopaedic surgery, against *Serratia marcescens* biofilm in vitro at doses of 10, 100, 1,000 times that of the established MIC for the planktonic isolate and found that the antibiotic, even at these concentrations, did not reduce biofilm biomass [12].

Reiter et al. tested rifampicin and vancomycin against methicillin-resistant *S. aureus* planktonic and biofilm isolates in vitro and found 32-32,000 times increase in resistance for rifampicin and 8-512 times increase in resistance for vancomycin in biofilm isolates [13]. They subsequently concluded that the tested antibiotic were not able to eradicate mature biofilm at the concentrations needed for planktonic microbes (the MIC).

Ruppen et al. tested gentamicin as an adjuvant to penicillin in *Group B Streptococcus* biofilm in vitro, and found a 2,000-4,000 times increase in resistance for penicillin in the presence of biofilm and 1-4 times increase for gentamicin [14]. The authors noted that the gentamicin doses tested did not correlate with achievable in vivo concentrations. The authors concluded that the MIC did not correlate to the susceptibility to the tested biofilm strains.

Hajdu et al. tested an array of antibiotics against *Staphylococcus epidermidis* biofilm in vitro. The planktonic bacteria susceptibilities were tested to all antibiotics in the study. When biofilm-indwelling bacteria was tested, susceptibilities were up to 128-times the established MIC. Only ceftriaxone showed a minor reduction in total biofilm biomass. No eradication occurred for any antibiotics at any level above MIC; it was also noted that these levels were much higher than any clinical in vivo achievable concentration [15].

Ravn et al. tested dislodged biofilm from in vitro implant infections of *S. aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Cutibacterium acnes* and found antimicrobial susceptibility to be identified at 4 times that of MIC (for *Escherichia coli* and ciprofloxacin) to 1.024 times that of MIC (for staphylococcus species + *Cutibacterium acnes* and vancomycin) [16]. The authors concluded that MIC correlation to in vivo values may not affect biofilm-indwelling bacteria.

Monzón et al. tested *Staphylococcus epidermidis* biofilm susceptibility on an array of antibiotics in vitro. All the isolates tested were fully susceptible to vancomycin in their planktonic form. The authors found that vancomycin, teicoplanin, clindamycin and oxifloxacin at MIC had a low killing rate in 24-hour mature biofilm. Rifampicin was not affected by the presence of mature biofilm and remained with a high killing rate at MIC [17]. The authors concluded that antibiotics may lose their killing ability in mature biofilm at clinical relevant in vivo levels, despite being fully susceptible at MIC.

Molina-Manso at el. tested susceptibility of Staphylococcus species biofilm in vitro and found that none of the tested antibiotics (including rifampicin, vancomycin, clindamycin, cloxacillin, ciprofloxacin) could eradicate the biofilm-indwelling bacteria, even at concentrations highly above the established MIC for the individual isolates [18].

Claessens et al. tested the effect of antibiotic concentration at up to 40 times the established MIC of the individual isolates in *Staphylococcus epidermidis* biofilm in vitro and found that only rifampicin could decrease but not eradicate the biofilm mass, whereas vancomycin, teicoplanin and oxacillin did not decrease the biofilm mass [19].

Given the plethora of evidence detailed above, there is a clear need to seek alternative approaches to the prevention and treatment of biofilm related infections. The use of local antibiotic delivery systems is widely regarded as a possible means to achieve sufficiently high concentrations of antibiotic to exceed the MBEC. However, there is little guidance on the optimal duration that MBEC should be exceeded to affect a cure. There is also concern that, although early elution of antibiotic from cement produces high local concentrations of antibiotics, late sub-MIC concentration may promote the development of antibiotic resistance, particularly amongst persister populations. Furthermore, the MBEC may well change with time of exposure to antimicrobials further complicating the determinants of optimal local dosage and carrier systems [20].

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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