QUESTION 11: What is the relevance of Minimum Inhibitory Concentration (MIC) of infecting organisms in biofilm-mediated chronic infection?

Authors: Jeppe Lange, Matthew Scarborough, Robert Townsend

Response:

The use of Minimum Inhibitory Concentration (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)

Post Meeting Rationale:

A literature search using *Biofilm* and *minimum inhibitory concentration* was performed using PUBMED and EMBASE, from inception to 8th February 2018. Further snowballing of references in acquired full text articles were performed. Titles screened, and if found appropriate, the abstract evaluated for acquisition of full text articles. A narrative approach was used in the screening process. Original papers as well as reviews were obtained. Only full text articles in English or German were reviewed.

Established methodologies for determining Minimum Inhibitory Concentrations (MIC) relate to the planktonic state of the bacteria, but not to biofilm-indwelling bacteria [1]. MIC is not suitable in predicting the effect of an antibiotic for a biofilm infection [6 7]. 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]. 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 orthopedic implant-associated biofilm infections [2,3].

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 latter may constitute up to 10% of biofilm. Post et al. showed that, although it was possible to eradicate biofilm caused by *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 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 not only prevents antimicrobial diffusion, but also MIC values alone cannot accurately determine the exact susceptibility of bacterial biofilms.

Ray et al. tested ceftriaxone and gentamicin against *Serratia marcescens* biofilm *in vitro* at doses of 10, 100, 1000 times that of the established MIC for the planktonic isolate, and found that, even at these concentrations, these antibiotics did not reduce biofilm biomass [12].

Reiter et al. tested rifampicin and vancomycin, against Methicillin Resistant *Staphylococcus aureus* planktonic and biofilm isolates *in vitro*, and found (32-32000) and (8-512) times increase in resistance, respectively, in biofilm isolates. They concluded that the tested antibiotic were not able to eradicate mature biofilm at the concentrations needed for planktonic microbes [13].

Ruppen et al. tested gentamicin as an adjuvant to penicillin in *Group B Streptococcus* biofilm *in vitro*, and found a 2000-4000 times increase in resistance for penicillin in the presence of biofilm, and 1-4 times increase for gentamicin. The gentamicin doses tested did not achieve similar concentrations *in vivo* and the MIC did not correlate to the susceptibility to the tested biofilm strains[14].

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 *Staphylococcus 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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