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In vitro antibiotic efficacy against staphylococci embedded in a biofilm involved in prosthetic joint infections: A MBEC problem?

Sir,

We read with great interest in a recent issue of the *International Journal of Antimicrobial Agents* the report by Molina-Manso et al. on the in vitro susceptibility to nine antibiotics of staphylococci isolates recovered from orthopaedic infections [1]. All antibiotics tested are commonly used in the treatment of prosthetic joint infections (PJIs).

The authors revealed that none of the antibiotics proved to be totally effective against biofilms with regard to minimum biofilm eradication concentration (MBEC) data, even with concentrations highly above the minimum inhibitory concentration (MIC) of the isolates. These results were obtained with the static Calgary Biofilm Device (CBD) method, which allows determination of the MBEC, which is the concentration of antibiotic killing nearly 100% of bacteria in a biofilm. Unfortunately, this method does not detect the actual number of bacteria in the biofilm used as an inoculum in MBEC tests. As inoculum size and (extended) incubation time (24 h) influence the results of susceptibility test challenge, MBEC values could be mistaken [2].

Despite these drawbacks, and although this method needs well-trained technicians, these data are interesting especially because the authors reported and confirmed the best activity of rifampicin (even if the results were poor with this system), the pivotal antibiotic used to eradicate staphylococci PJIs. None of the antibiotics was effective in eradicating the biofilm but, at 24 h, with this system rifampicin remains a key antibiotic against Gram-positive pathogens [3]. In this study, Molina-Manso et al. could not identify any difference in antibiofilm activity. We agree with the authors that all of the strains tested were able to form biofilms on the CBD pegs but in their conditions.

According to our experience, whatever the system used to detect biofilm or adherence abilities with the BioFilm Ring Test, *Staphylococcus aureus* clinical isolates involved in hip and knee PJIs can produce biofilm. Nevertheless, the comparison remains delicate because this last method investigates the first step of adhesion in the biofilm cycle life [4]. In contrast, the CBD method as well as the Christensen or resazurin methods are considered as endpoint methods. Thus, we need to keep in mind that technical conditions, especially the medium used, pH, oxygenation, ionic concentrations and glucose concentration, could lead to huge variations in biofilm determination that do not mimic real life. Therefore, these MBEC results should be interpreted with caution according to the limits and performance of the method used [4].

Second, we were surprised to find a slight activity of tigecycline, a bacteriostatic antibiotic. According to personal data and the literature, with time–kill curve and biofilm studies, tigecycline confirmed its bacteriostatic activity. Moreover, with an in vitro MBEC determination method using microcalorimetry [5], we systematically did not observe any efficacy against an early or mature biofilm but only a delay in the time of growth. It would have been interesting to compare the MIC and minimum bactericidal concentration in logarithmic and stationary phase with the MBEC determination obtained with this system.

Third, we agree with the authors that the first step of PJI treatment remains excellent surgery (decrease in the inoculum) to cure the patients. PJI requires a rapid and accurate diagnosis to limit biofilm maturation, the development of small-colony variants, and invasion of osteoblasts, especially with *S. aureus* strains leading to persistence and chronicity. Numerous recent clinical or research studies revealed the excellent combination of different drugs with excellent tolerance such as rifampicin, daptomycin, ciprofloxacin, fosfomycin or trimethoprim/sulfamethoxazole [3]. These clinical data do not correlate with the in vitro resistance level revealed in this study.

To conclude, in vitro biofilm antibiotic efficacy determination remains difficult to interpret and extrapolate in routine. The correlation between in vitro and in vivo data remains debatable. In vivo animal models could help in the therapeutic strategy. Nevertheless, further standardised studies are clearly needed to achieve a best understanding of antibiotic antibiofilm activity.

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High-dose tigecycline-associated alterations in coagulation parameters in critically ill patients with severe infections

Sir,

Tigecycline, a glycylcycline antimicrobial agent with a broad spectrum of in vitro activity against a variety of Gram-positive and Gram-negative aerobic and anaerobic bacteria, has been approved to treat complicated intra-abdominal and skin and skin-structure infections as well as community-acquired bacterial pneumonia at a loading dose of 100 mg, followed by 50 mg twice daily [1]. How-

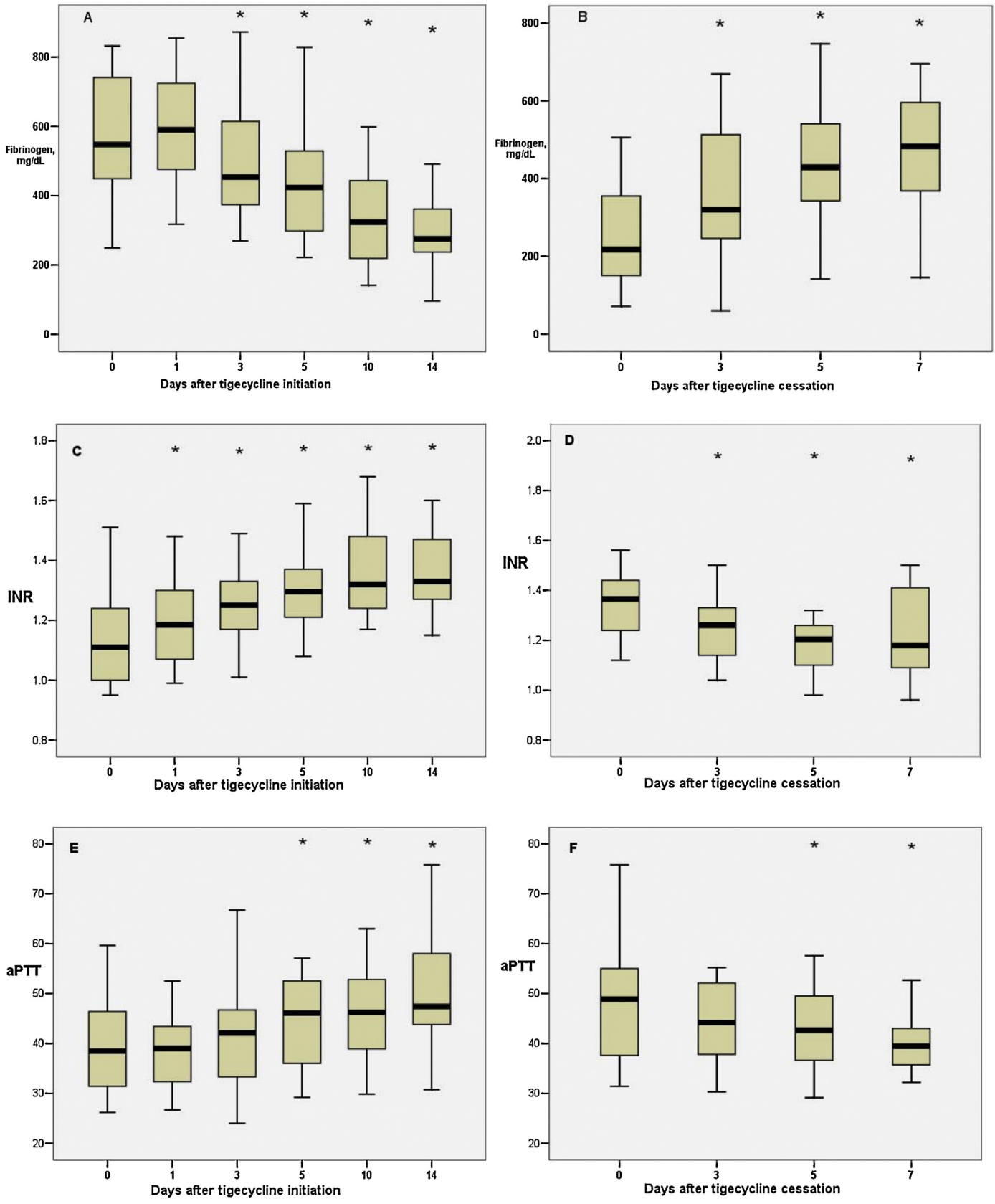

Fig. 1. (A and B) Plasma fibrinogen concentration, (C and D) international normalised ratio (INR) and (E and F) activated partial thromboplastin time (aPTT) after tigecycline initiation (left) and cessation (right). Horizontal bars represent the median value, boxes represent the interquartile range and whiskers indicate the 5th and 95th percentiles. Friedman's test *P*-value for each parameter was <0.001. $^{*}P < 0.05$ vs. Day 0, paired Wilcoxon's test.