O065
Oral Session
Basic science: pathogenesis and epidemiology of Gram-positive bacteria

ANTIMICROBIAL ACTIVITY AGAINST INTRA-OSTEOBLASTIC STAPHYLOCOCCUS AUREUS: A NEW THERAPEUTIC CONCEPT FOR BONE AND JOINT INFECTION?

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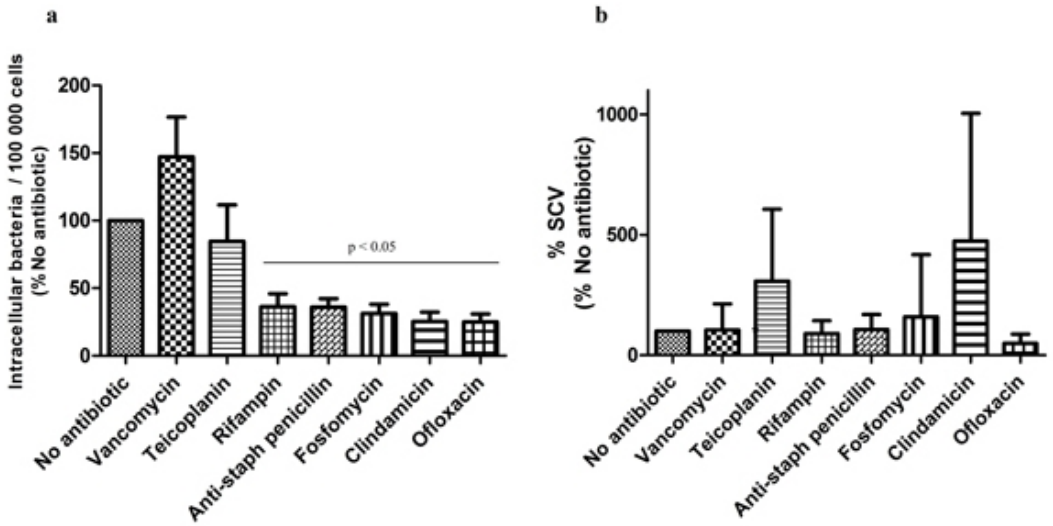
Objectives: To date, the choice of antimicrobial therapy for *S. aureus* bone and joint infection (BJI) is based on the intrinsic antibacterial activity and bone penetration of the available antibiotics. Recently, some pathophysiological mechanisms of BJI have been also taken into consideration, which lead to recommend the use of rifampin in orthopaedic device-associated infections due to its activity against staphylococcal biofilm for example. While *S. aureus* can be internalized into human osteoblasts and persist into bone cells partly as small colony variants (SCV), the intracellular activity of antimicrobials is not currently considered in treatment strategies of BJI, although this intracellular bacterial reservoir can lead to BJI chronicity and relapse. We aimed to evaluate the intra-osteoblastic activity of the main antimicrobials used for staphylococcal BJI in an *ex vivo* model of osteoblast infection, and to assess their impact on the emergence of intracellular SCV.
Methods: Human osteoblastic MG63 cells were infected for 2h with HG001 *S. aureus*. After selective killing of extracellular bacteria with lysostaphin, infected cells were incubated for 24h with oxacillin, clindamycin, fosfomycin, ofloxacin, rifampin, vancomycin or teicoplanin, using intraosseous concentrations reach in humans with standard therapeutic dosages. All intracellular bacteria and SCV were then quantified by plating cell lysates, using a standardized image analysis method (colonies were considered as SCV if their surface were <5% of the median area of all colonies). Cellular toxicity of the tested antibiotics was assessed using a lactate deshydrogenase (LDH) release assay.
Results: Compared with untreated cells, a significant decrease in intracellular inoculum was observed with rifampicin (-63.8% [95%CI 54.1-73.4]), oxacillin (-64.0% [57.7-70.4]), fosfomycin (-68.8 [61.9-75.7]), clindamycin (-74.6 [67.8-81.4]) and ofloxacin (-74.8 [69.1-80.6]), with no antibiotic-related cell toxicity (figure 1a). Glycopeptides had no activity against intracellular staphylococci. A trend in intracellular SCV increase was observed with teicoplanin and clindamycin, while other molecules had a neutral effect (figure 1b).



Conclusions: Our study provides the first evaluation of the intra-osteoblastic activity of a large panel of antistaphylococcal antibiotics used at human bone concentration. Regarding the eradication of the staphylococcal intracellular reservoir, the most effective molecules appeared to be ofloxacin and clindamycin. Ofloxacin seemed to be the only molecule with a satisfactory intracellular activity while limiting the emergence of SCV. These data provide a basis for refining the choice of antibiotics to

prioritize in the management of difficult-to-treat BJI. For instance, our results justify the combination of an anti-biofilm molecule, such as rifampin, with a fluoroquinolone for its intracellular activity.