INTRODUCTION: In the fields of traumatology and orthopedics, staphylococci are the most frequently isolated pathogens. Notably *Staphylococcus aureus* and *S. epidermidis* are known to be the major causative agents of the bone disease osteomyelitis. Once osteomyelitis has occurred in fractured bones, removal of implants is necessary followed by multiple debridements. In addition osseous infections can be reduced by the use of local and systemic antibiotics, but nevertheless, the clinical relevance of osteomyelitis remains an unsolved problem. Patients can have recurrent attacks of osteomyelitis after completion of treatment, even when causative organisms cannot be isolated. At worst the infection persists for decades with sporadic recurrence.

For local release of antibiotics, currently foreign bodies like antibiotics loaded bone cements, antibiotics impregnated collagen sponges and polymethylmethacrylate beads are implanted and particular removed later on. The most widely applied agents in local delivery systems are aminoglycosides like gentamicin.

Due to the fact *S. aureus* has the capability to avoid into the osteoblasts intracellular environment, a sufficient antibiotic treatment is getting difficult. This internalization may provide a reservoir of bacteria for recurring osteomyelitis. Aim of this study was to investigate the efficacy of gentamicin versus rifampicin in a primary osteoblast infection model.

METHODS: Primary human osteoblasts were routinely cultured in growth medium consisting of Minimum Essential Medium (Eagle) (MEM) and HAM’s F-12 (1:1), supplemented with 10% fetal calf serum (FCS), 100 U of penicillin ml\(^{-1}\) and 100 µg of streptomycin ml\(^{-1}\). All samples were obtained with permission of the local ethic committee. The assay medium was MEM/HAM’s F-12 (1:1), supplemented with 1% human serum albumine (HSA). Prior to assay, described elsewhere with minor modifications (1), bacteria (different *S. aureus* strains; also see Tab. 1) were grown overnight. Bacterial cell numbers were estimated spectrophotometrically at 540 nm. Bacteria were harvested by centrifugation and resuspended in 1 ml phosphate-buffered saline pH 7.3 (PBS) supplemented with 1% HSA to give 5 x 10\(^5\) CFU ml\(^{-1}\). Cells are seeded at 10\(^5\) per well into 12-well tissue cultures plates in 1 ml of growth medium. Accordingly, they were cultured for 2 days until they were confluent; at the first day of assay, cells were washed twice with 1 ml MEM/HAM’s F-12 and then incubated with 1 ml of assay medium. 40 µl of the bacteria culture with an OD\(_{540}\) = 1 (= 5 x 10\(^5\) CFU ml\(^{-1}\)) were added to the osteoblasts, incubated for 30 minutes at 25°C to allow sedimentation and then shifted to 37°C in a 5% CO\(_2\) incubator for 3 h. After the 3 hours coculture of bacteria (multiplicity of infection [MOI] of 100:1) external *S. aureus* were distrusted by 20 µg ml\(^{-1}\) lysostaphin. To determine success of internalization, cells were washed subsecutively with 1 ml of PBS to remove non internalized bacteria, stripped of the wells by adding 0.2 ml 0.05% Trypsin containing 0.02% EDTA and stopped with 0.8 ml MEM/HAM’s F-12 and 10% FCS. After washing twice with PBS, bacteria were harvested via cell lysis by adding 1 ml aqua dest. Enumeration of colony forming units (CFU) was performed by serial dilution and plate counting on Mueller-Hinton agar plates. Alternativley after the lysostaphin treatment, osteoblasts were washed twice with 1 ml of MEM/HAM’s F-12 to remove non internalized bacteria, 1 ml of fresh growth medium containing gentamicin (10 µg ml\(^{-1}\)) or rifampicin (7 µg ml\(^{-1}\)) instead of penicillin/streptomycin was added, and the cultures were incubated for up to 20 hours. Internalization of *S. aureus* was quantified as described above. The invasiveness of Cowan I was set at 100% (1).

RESULTS: In order to investigate the efficacy of gentamicin a standardized primary human osteoblasts infection model was established. Internalization of different *S. aureus* strains and a negative control (TM300) by this cells showed an increase of 85 % for ATCC 49230 in comparison to Cowan I (p = 0.018) after 4 hours (Fig. 1). After 20 hours ATCC 49230 showed in presence of gentamicin a non significant increase of internalization (25 % in comparison to Cowan I (p = 0.216)). In cells treated with rifampicin a significantly decreased internalization of Cowan I (p = 0.02) and ATCC 49230 (p = 0.003) became apparent in comparison to gentamicin treated cells (Fig. 2).

### DISCUSSION:

In this study a standardized cell culture infection model for primary osteoblasts was established. As described in literatur, different strains of *S. aureus* posses variable abilities to invade osteoblasts. The ATCC 49230 strain is frequently used for *in vivo* infection models (2), however little is known about its internalization by osteoblasts into their intracellular environment. It could proved that the rate of internalization of ATCC 49230 compared to Cowan I, used as reference (1), is 85 % higher. A comparison of gentamicin versus rifampicin treatment of infected osteoblasts exposed a significantly reduced internalization in presence of rifampicin after 20 hours. The use of gentamicin as standard antibiotic for local treatment of osseous infections in everyday clinic needs to be reconsidered.

In further studies combinations of antibiotics will be investigated using this infection model. With sub inhibitory concentrations of locally applied gentamicin the mechanism that leads to the appearance of small colony variances of *S. aureus* can be examined.

### REFERENCES:


**AFFILIATED INSTITUTIONS FOR CO-AUTHORS:**

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### Table 1: Strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Properties</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> Cowan 1</td>
<td>NCTC 8530 (isolated from septic arthritis)</td>
<td>ATCC 12598</td>
</tr>
<tr>
<td><em>S. aureus</em> Rosenbach</td>
<td>CDC 587 (isolated from chronic osteomyelitis)</td>
<td>ATCC 49230</td>
</tr>
<tr>
<td><em>S. aureus</em> ST239, 635/93</td>
<td>Wild-type isolate, MRSA</td>
<td>W. Witte, Wernigerode, Germany</td>
</tr>
<tr>
<td><em>S. carnosus</em> TM300</td>
<td>Wild-type</td>
<td>ATCC 51365</td>
</tr>
</tbody>
</table>

**Fig. 1:** Comparison of internalization of *S. aureus* Cowan 1, ST239, ATCC 49230 and *S. carnosus* TM300 by primary human osteoblasts after 4 hours. Data are from a representative experiment performed at least three times in triplicates.

**Fig. 2:** Comparison of internalization of *S. aureus* Cowan 1, ATCC 49230 and *S. carnosus* TM300 by primary human osteoblasts after 20 hours incubation with gentamicin or rifampicin. Data are from a representative experiment performed at least three times in triplicates.

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