Ampicillin plus Ceftriaxone Combination for *E. faecalis*

Orthopedic Infections. A Pilot Study

G. Euba1*, J. Lora-Tamayo1, O. Murillo1, S. Pedrero2, J. Cabo2, R. Verdaguer3, J. Ariza1

1Infectious Diseases Department, 2Orthopedic Surgery Department, 3Microbiology Department

IDIBELL. Hospital Universitari de Bellvitge. Barcelona (Spain)

Keywords: *E. faecalis*; orthopedic infections; combination therapy; ampicillin-ceftriaxone

Running title: AMP-CRO combination in *E. faecalis* orthopedic infection

* Corresponding author:

Gorane Euba, MD
Infectious Diseases Department
Hospital Universitari de Bellvitge
Feixa Llarga s/n
08907 - L'Hospitalet de Llobregat (Barcelona) - SPAIN
Tel: +34 93 260 7625 / Fax: +34 93 260 7637
goranee@bellvitgehospital.cat
ABSTRACT

Serious *E. faecalis* infections usually require combination therapy to achieve bactericidal effect. In orthopedic infections, the prognosis of enterococcal etiology is considered poor and the use of aminoglycosides questioned. Ampicillin–ceftriaxone combination has recently been accepted as alternative therapy for enterococcal endocarditis. After one of our patients with endocarditis and vertebral osteomyelitis was cured with ampicillin-ceftriaxone, we started a pilot study in orthopedic infections. Patients with infections due to *E. faecalis* (≥2 surgical samples or blood cultures) diagnosed during 2005-2008 were recruited. Polymicrobial infections with ampicillin and ceftriaxone resistant microorganisms were excluded. Patients received ampicillin 8-16 g/day + ceftriaxone 2-4 g/day and were followed up prospectively. Of 31 *E. faecalis* infections, 10 received ampicillin-ceftriaxone. Including the first patient, 11 cases were treated with ampicillin-ceftriaxone: 3 were prosthetic joint infections, 3 instrumented spine arthrodesis, 2 osteosynthesis devices, 1 foot osteomyelitis and 2 vertebral osteomyelitis with endocarditis. Six (55%) were polymicrobial. All except the vertebral osteomyelitis cases required surgery, with retention of foreign material in 6. Ampicillin-ceftriaxone was given for 25 days (interquartile range 15-34), followed by amoxicillin in 7 (64%). One patient with endocarditis died within 2 weeks (hemorrhagic stroke) and was not evaluable. In 1 with prosthesis retention the infection persisted; 9/10 (90%) were cured but 1 was superinfected. Follow-up was 21 months (interquartile range 14-36). Ampicillin-ceftriaxone may be a reasonable synergistic combination to treat *E. faecalis* orthopedic infections. Our experience, though limited, shows good outcome and
tolerability and may provide a basis for further well designed comparative studies.
INTRODUCTION

E. faecalis is a low virulence microorganism that colonizes the human gastrointestinal tract (23) and produces a variety of infections especially under antimicrobial pressure or in the nosocomial setting: urinary tract and intraabdominal infections, bacteremia, endocarditis, meningitis and also orthopedic and foreign body-related infections (20). In orthopedic infections, enterococci are relatively common etiologic agents (26, 27); however, it is often difficult to distinguish infection from colonization, as they may be isolated in samples of doubtful significance or in combination with other microorganisms.

As is well known in clinical practice, some enterococcal infections are difficult to treat (21). Though susceptible at relatively low MIC values, enterococci are characteristically resistant to the bactericidal effect of cell wall-active antibiotics (16). Most E. faecalis strains show the “paradoxical or Eagle effect”, in which penicillins are more bactericidal just above the MIC and less bactericidal as the drug concentration increases (6, 9). This phenomenon has been attributed by some authors to an intrinsic defect in the autolytic activity of the microorganism (14). As a result of these special features, in the absence of high level AG resistance, an ampicillin-AG combination is now the therapy of choice for deep-seated infections by E. faecalis where a bactericidal effect is desirable, such as endocarditis or meningitis (20, 23).

In orthopedic and foreign body infections, in which biofilm formation occurs, bactericidal effect is sought in order to eradicate infection and avoid relapses.
For serious enterococcal orthopedic infections, most authors recommend a combination therapy with AG (26, 37). However, the role of AG in the treatment of orthopedic infections has often been questioned, as the local conditions in infected bone may reduce their efficacy against susceptible microorganisms (17), and they have serious side effects that may limit their use.

Ampicillin-ceftriaxone combination (AMP-CRO) has recently been recommended (strength IIbC) for endocarditis due to *E. faecalis* highly resistant to AG (2) after the experience of Gavaldà *et al* (11-13). The basis for these reports was an *in vitro* study by Mainardi *et al* which found a synergistic effect between amoxicillin and low levels of cefotaxime against several AG susceptible and resistant *E. faecalis* strains (18).

Pyogenic vertebral osteomyelitis may present as a complication of infective endocarditis (22, 25, 32). We undertook this pilot study after one of our patients with enterococcal endocarditis and vertebral osteomyelitis was treated with AMP-CRO and cured at both infection sites. To our knowledge, this is the first study to evaluate a double β-lactam combination in the treatment of orthopedic infections caused by *E. faecalis*. 
PATIENTS AND METHODS

SETTING
The study was performed in a 900-bed tertiary care teaching hospital in Barcelona, Spain. Patients were attended in the Orthopedic Infection ward, a multidisciplinary section in which Orthopedic Surgery and Infectious Disease departments collaborate.

STUDY DESIGN
1) Inclusion and exclusion criteria
After approval of the proposal by the hospital’s ethical committee, patients diagnosed with *E. faecalis* orthopedic infection between January 2005 and January 2008 were sequentially recruited as eligible subjects for the study. Osteomyelitis and foreign body infection were diagnosed on a clinical basis (local pain, purulent draining), with complementary laboratory parameters (acute phase reactant elevation) and compatible imaging (plain radiographs, CT-scan or magnetic resonance). Endocarditis was diagnosed using Duke’s criteria. Spontaneous vertebral osteomyelitis was diagnosed by the presence of clinical symptoms (spinal pain and localized tenderness or limited range of motion) and characteristic image findings (in plain radiographs and CT-scan or magnetic resonance), as described elsewhere (8). Prosthetic joint infection was diagnosed and classified according to Tsukayama *et al* (31).
The following were considered exclusion criteria: AMP resistant *E. faecalis* strain, polymicrobial infection with microorganisms resistant to AMP-CRO combination and allergy to penicillin.

2) **Microbiological diagnosis**

The diagnosis of *E. faecalis* osteomyelitis required the isolation of *E. faecalis* in ≥2 surgical samples or in blood cultures. *E. faecalis* identification was performed using the automatic Dade Behring MicroScan system (Sacramento, California, USA). MICs were determined according to the Clinical and Laboratory Standard Institute guidelines (5) by a microdilution method using Sensititre Emiza panels (STAENC1F, Trek Diagnostic Systems Ltd, UK).

3) **Antibiotic therapy**

Cases treated with the AMP-CRO combination for at least 1 week within the global treatment schedule were considered evaluable. Antibiotic dosing varied depending on the site of infection, but a minimum of AMP 8 g/day + CRO 2 g/day was considered appropriate (37).

4) **Follow-up and outcome definitions**

The follow-up period is calculated from the end of antibiotic therapy until February 2009. During follow-up, patients were evaluated periodically for persistent or new clinical signs of infection. The patient was considered cured if the enterococcal infection was eradicated by the time of the last control. Persistence of the enterococcal infection was considered when clinical symptoms persisted and cultures were still positive for *E. faecalis*. Relapse was
defined as a temporary remission of the symptoms and later reappearance of
local inflammatory signs, pain or purulent draining with the isolation of *E.
faecalis* as in the previous episode. **Superinfection** was diagnosed when a
temporary remission of the symptoms was followed by reappearance of local
inflammatory signs and the isolation of a different microorganism.

5) *In vitro* time kill-curves

Twenty-four hour kill-curves were made in exponential growth and stationary
phases with 2 of the clinical isolates. Exponential growth phase studies were
performed using a tube macrodilution method in Mueller-Hinton Broth (MHB)
with inocula of $10^7$ CFU/ml and multiple antibiotic concentrations (AMP ranging
from 0.12 to 64 mg/l; CRO 5 and 10 mg/l). Quantitative bacterial counts were
determined as log CFU/ml at 6 and 24 h of incubation at 37ºC. To avoid the *in
vitro* carryover effect, the sample was allowed to be absorbed by the agar until
the plate surface appeared dry and was then spread over the plate. The
stationary phase studies were performed using bacteria at $10^8$ CFU/ml, which
were recovered from an overnight culture in trypticase soy broth, centrifuged
and resuspended in a nutrient-restricted medium (phosphate buffered saline +
1% glucose + 4% MHB), thus ensuring that bacteria remained stable for up to
24h under these conditions. Quantitative cultures were performed as described
above. Bactericidal activity was defined as a $>3$ log10 decrease from the initial
inoculum CFU/ml after 24 h.

The results of the combination were compared with the most active single drug;
synergy, indifference and antagonism were then defined as $\geq2$ log increase in
killing, <1 log change (increase or decrease) in killing and ≥2 log decrease in killing respectively.

AMP (Normon, Madrid, Spain) and CRO (Roche Farma, Madrid, Spain) purified powder were resuspended following the laboratory’s recommendations.

STATISTICAL ANALYSIS
A descriptive analysis of data was performed with SPSS software version 15.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables are expressed with the median and range or interquartile range (IQR).
RESULTS

Clinical results

In the study period, 31 orthopedic infections by *E. faecalis* were diagnosed. None of the *E. faecalis* strains was AMP-resistant. Twenty-five (80%) had polymicrobial infection. The following were excluded: 16 due to polymicrobial infection including an AMP-CRO resistant microorganism (mostly staphylococci and *P. aeruginosa*), 1 due to penicillin allergy and 4 in which the physician decided to give penicillin monotherapy. Finally, 10 of these patients were treated with AMP-CRO.

Including the first case with endocarditis and vertebral osteomyelitis treated before 2005, 11 patients received AMP-CRO. The clinical data are summarized in table 1. Five patients (45%) were male and median age was 69 years (range 24-83). Six had (55%) polymicrobial infection: four with *E. coli*, 1 with *P. mirabilis* and 1 with Lactobacillus sp.

The sites of infection were the following: 3 prosthetic joint infections (27%) (1 located in the hip and 2 in the knee); 3 instrumented spine arthrodeses (27%), 2 of them with additional meningitis due to postsurgical cerebrospinal fluid leak; 2 osteosynthesis devices (18%); 1 osteomyelitis of the foot (9%) and 2 vertebral osteomyelitis with endocarditis (18%). With the exception of the patients with spontaneous vertebral osteomyelitis, all underwent surgery (median 1 surgery, range 1-3). Among the patients with meningitis, patient 7 required the
placement of a lumbar catheter for 7 days in order to allow cerebrospinal fluid fistula to heal and patient 9 was cured with debridement and antibiotics only.

In 8 cases (72%) a foreign body was involved, which was retained in 6 and removed in 2. Among the prosthetic joint infections (patients 2, 3 and 4), two were late chronic infections and the other one (patient 2) was early postoperative with stability of the implant. The latter could be managed with debridement only. In case 3 the acetabular component was exchanged in one-stage and the femoral component was retained, with successful outcome. Though the complete removal of the knee prosthesis was indicated in case 4, the procedure proved impossible due to technical problems. As a consequence, the intramedullary component of the prosthesis, which was involved in the infection, had to be retained. This patient was given suppressive antibiotic therapy (long-term oral antibiotic therapy given only to control clinical symptoms, not to eradicate infection (27, 37)).

The AMP dose ranged between 8 and 16 g/day and the CRO dose between 2 and 4g/day. The median duration of AMP-CRO treatment was 25 days (IQR 15-34). Sequential therapy with amoxicillin was given in 7 cases (64%) (suppressive in one). None of the patients developed any side effects with the protocol treatment.

Median follow-up was 21 months (IQR 14-36). Patient 11 (endocarditis) died within 2 weeks due to a hemorrhagic stroke and was not evaluable for the outcome of the osteomyelitis. In 9/10 (90%) enterococcal infection was
eradicated, but 1 was superinfected by *S. aureus*. Only in the case of patient 4 did the infection persist; finally, this patient required the complete removal of the foreign material by a supracondylar amputation to eradicate infection.

**In vitro results**

*E. faecalis* strains from patients 3 and 4 were recovered for further *in vitro* studies. The MICs of the strain from patient 3 (cured) for AMP and CRO were 1 and >128 mg/l, and for the strain from patient 4 (persistence) 0.5 and >128 mg/l respectively.

Exponential phase kill-curves for the strains from patients 3 and 4, showed bactericidal effect with AMP alone ranging between 1 and 8 mg/l and 0.5 and 8 mg/l respectively. In both cases, concentrations higher than 8 mg/l were less active (the Eagle effect). For both strains, AMP-CRO combination using CRO 5 and 10 mg/l achieved a synergistic effect and the bactericidal window was extended to AMP concentrations between 0.12 and 8 mg/l (figure 1).

Stationary phase studies did not achieve any bactericidal activity, either with AMP alone or with AMP-CRO combination at any concentration, and no synergy was observed for either of the strains tested (figure 2).
DISCUSSION

This pilot study presents promising results regarding the clinical efficacy and bactericidal activity of the AMP-CRO combination in the treatment of osteoarticular infections.

The significance of the presence of *E. faecalis* in clinical samples of orthopedic infections and whether the prognosis in these cases is good or bad, remain controversial issues. Nevertheless, as the process of eradicating *E. faecalis* from a foreign body or a focus of osteomyelitis is complex, it has been described as a difficult-to-treat organism (37), probably due to the difficulty of achieving bactericidal effect with the antimicrobial agents available.

Classically, bactericidal effect has been hypothetically provided by a penicillin-AG combination, the current therapy of choice, as a consequence of the extended experience in the treatment of enterococcal endocarditis. However, it is well known that the synergistic bactericidal activity of AG may be highly compromised in the setting of orthopedic infections with local purulence, acidic pH and anaerobic conditions. Also, as in the case of the treatment of enterococcal endocarditis, the serious side effects of AG discourage their use in prolonged treatments or fragile patients; thus, many cases of orthopedic infections due to *E. faecalis* are finally treated with therapies that are known to be only bacteriostatic. A recent retrospective study of prosthetic joint infections found a higher rate of adverse side effects in the group of patients treated with AG combination compared with those on β-lactam monotherapy; moreover, the
group that received combination therapy did not have a better outcome (7). In this study, patients were mainly managed with prosthesis removal and the authors concluded that bacteriostatic therapies might be enough to treat those cases, in combination with aggressive surgery, surely meaning prosthesis removal. However, aggressive surgery is not always advisable or possible. In prosthetic joint infections classified as early postoperative or hematogenous according to Tsukayama (31), most authors agree in recommending debridement and prosthesis retention (conservative surgery) when the duration of symptoms is short and the implant is stable (37). In addition, the infections affecting osteosyntheses that stabilize fractures may require intense antibiotic therapy until the consolidation of the fracture allows the implant removal. In these situations managed with conservative surgery, it is natural to think that antimicrobial therapies with bactericidal effect might improve the evolution of enterococcal infection compared to the bacteriostatic treatments, in agreement with classical recommendations.

Our patients mainly correspond to this group of cases managed medically or with conservative surgery, including 2 with vertebral osteomyelitis and 6 out of 8 with retention of the foreign body. The isolation of *E. faecalis* in repeated samples highlighted the clinical role of this microorganism in these cases, even in those with polymicrobial isolates. The frequency of polymicrobial etiology in enterococcal osteoarticular infections has been noted previously (26). Therefore, it is not surprising that half of the patients on the protocol treatment and many of those excluded had polymicrobial infection. Many of the cases excluded were infections with *S. aureus* and/or *P. aeruginosa*, very frequent
pathogens in nosocomial infections that could not be treated with the AMP-CRO combination.

In recent years, the double β-lactam combination has opened up a new possibility for the treatment of *E. faecalis* endocarditis. Mainardi *et al* described that low concentrations of amoxicillin and cefotaxime were synergistic against AG susceptible and resistant *E. faecalis* strains compared with amoxicillin alone, and tentatively attributed this synergy to fact that each drug had different PBPs as targets (amoxicillin partially saturated PBPs 4 and 5 and cefotaxime PBPs 2 and 3), thus improving the bactericidal effect (18). In further studies, Gavaldà *et al* reported that the AMP-CRO combination was synergistic *in vitro* and also presented good results *in vivo* with *E. faecalis* experimental endocarditis in rabbits (12, 13). These studies gave rise to a multicenter prospective non-controlled study in a cohort of patients with endocarditis due to *E. faecalis* highly resistant to AG, and patients with endocarditis due to AG susceptible strains but at risk of nephrotoxicity related to AG use (11). The AMP-CRO combination achieved promising results, being curative in 71.4% of the cases in the group with strains highly resistant to AG and in 63.6% of the cases in the AG susceptible group.

In our *in vitro* studies, the exponential growth phase kill-curves corroborated previous reports (13, 18) that the AMP-CRO combination was synergistic. An extension of the bactericidal window was found compared with AMP alone in the range of sub-MIC AMP concentrations. This was confirmed in both of the strains recovered from two representative patients in our series: one who was
cured with retention of some foreign material (patient 3) and one in whom the infection persisted (patient 4). Therefore, the clinical failure in this patient could not be attributed to a lack of synergy against the strain. Though the clinical relevance of this *in vitro* synergy is difficult to determine, it is logical to think that prolonging the time of bactericidal antibiotic concentration in the infection site may improve bactericidal activity. In contrast, in our stationary phase experiments, neither AMP monotherapy (at all concentrations tested) nor the combination were bactericidal, and no synergy was observed with the addition of CRO. There is no mention of stationary bacteria in previous studies; to our knowledge, the effect of β-lactams on *E. faecalis* in the stationary phase has not been reported before. This is an important point, because the ratio between the area under the curve (AUC) and the minimal bactericidal concentration (MBC) of stationary or adherent bacteria has been reported to be the most reliable marker of antibiotic efficacy in foreign body infections (1, 34, 36). Endocarditis and orthopedic infections are characterized by the presence of bacterial biofilms (3), where bacteria express variable phenotypic tolerance to antimicrobials (4). However, this loss of killing activity against biofilm-forming bacteria is not homogeneous among the different microorganisms and antimicrobials. Though the efficacy of β-lactams and AG in biofilms is strongly reduced (28), streptococcal and enterococcal osteoarticular infections have traditionally been managed with these antibiotics, which are considered the treatment of choice. Today, the exact *in vitro-in vivo* correlation in the treatment of these infections is difficult to determine.
Literature on enterococcal osteoarticular infection is heterogeneous and provides variable outcome data. The cure rates reported with cell wall-active antibiotic monotherapies range between 33 and 88%, and between 67 and 100% using AG combinations (7, 26, 30, 32). In our small clinical series, the AMP-CRO combination was well tolerated and the cure rate achieved at the last follow-up was 90%, despite the fact that most patients were managed with retention of the foreign material. The only case that failed was a patient with a late chronic infection of a knee arthroplasty in whom the treatment of choice (complete prosthesis removal) could not be performed for technical reasons.

Regarding the AMP-CRO doses used in this study it is worth noting some pharmacokinetic (PK) and pharmacodynamic considerations. In the case of β-lactams it is generally recognized that time above the MIC is the best predictor of efficacy, but no special attention has been paid to the minimum free-drug time above MIC required for efficacy, perhaps because of the low protein binding of these antibiotics and the safety of using high doses. In fact, this parameter has not been clearly defined in animal models of enterococcal infection, and the possible negative consequences of using too high doses of AMP due to the Eagle effect in vivo are not known. In any case, considering a protein binding of about 17% (19), 2 g of AMP should provide free serum concentrations above the MIC of *E. faecalis* [mean MIC 1 mg/l (range 0.5-4)] (19, 23) for most of the 6-hour interval (10).

In the case of CRO, which is inactive individually against *E. faecalis* (MIC range 1->128) (15, 33), the high and concentration dependent protein binding
contributes to its unique PK properties (24, 29). Nonetheless, the previous experimental studies by Gavalda et al. (12, 13), the only ones published about AMP-CRO combination for enterococcal infection, did not refer to free drug, therefore it is difficult to establish a relation to the reports focused specifically on human PK of CRO (35). After considering all available data, we think that a daily dose of 2g of CRO may be sufficient to maintain concentrations of 5-10 mg/l for a long interval at the site of infection, the required minimum concentrations that provide synergistic effect with sub-MIC AMP concentrations. Based on the literature on enterococcal endocarditis, we believe that the AMP–CRO combination may be a reasonable synergistic alternative to the classical AMP-AG combination for the treatment of osteomyelitis and foreign-body infections due to *E. faecalis*. The synergy of the double β-lactam combination against *E. faecalis* is reproducible *in vitro* in experiments representing planktonic bacteria, though no benefit against stationary bacteria has been demonstrated so far. The favorable outcome and good tolerability observed in our small series may provide a basis for further well-designed comparative studies.
ACKNOWLEDGEMENTS

None of the authors have any conflict of interests. We thank Michael Maudsley for revising the English manuscript. These data were reported partially at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Washington D.C., 2008 (K-546). G. E. was supported by a grant from the Instituto de Salud Carlos III (FI05/00438). This study was supported by the Spanish Network for the Research in Infectious Diseases (grants REIPI C03/14 and REIPI RD06/0008).
REFERENCES


Ariza. 2008. Long-term clinical and radiological magnetic resonance imaging outcome
of abscess-associated spontaneous pyogenic vertebral osteomyelitis under
to Enterococcus faecalis using once-daily dosing regimen of gentamicin plus simulated
11. Gavalda, J., O. Len, J. M. Miro, P. Munoz, M. Montejo, A. Alarcon, J. de la Torre-
Romeu, F. Marco, C. Torres, P. Tornos, A. Planes, V. Falco, B. Almirante, and A.
Pahissa. 2007. Brief communication: treatment of Enterococcus faecalis endocarditis
with ceftriaxone and gentamicin in the treatment of experimental endocarditis due to
Enterococcus faecalis with no high-level resistance to aminoglycosides. J Antimicrob
Efficacy of ampicillin plus ceftriaxone in treatment of experimental endocarditis due to
Enterococcus faecalis strains highly resistant to aminoglycosides. Antimicrob Agents
bacteria. Rev Infect Dis 7:368-86.
in vivo activities of LB10522, a new catecholic cephalosporin. Antimicrob Agents


<table>
<thead>
<tr>
<th>Patient</th>
<th>Infection Site</th>
<th>Bacteremia</th>
<th>Etiology</th>
<th>Surgery</th>
<th>IV therapy</th>
<th>AMP-CRO</th>
<th>Sequential PO therapy</th>
<th>Follow up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Endocarditis</td>
<td>+</td>
<td>E. faecalis</td>
<td>None</td>
<td>47d</td>
<td>29 d</td>
<td>None</td>
<td>50 mo</td>
<td>Cured</td>
</tr>
<tr>
<td>2</td>
<td>Knee PJI (Early)</td>
<td>-</td>
<td>E. faecalis</td>
<td>Debridement</td>
<td>43 d</td>
<td>25 d</td>
<td>AMX 61 d</td>
<td>26 mo</td>
<td>Cured</td>
</tr>
<tr>
<td>3</td>
<td>Hip PJI (Late)</td>
<td>-</td>
<td>E. faecalis</td>
<td>Partial exchange (1 stage)</td>
<td>46 d</td>
<td>42 d</td>
<td>AMX 16 d</td>
<td>14 mo</td>
<td>Cured</td>
</tr>
<tr>
<td>4</td>
<td>Knee PJI (Late)</td>
<td>-</td>
<td>E. faecalis</td>
<td>Debridement &amp; partial removal</td>
<td>58 d</td>
<td>16 d</td>
<td>AMX ST</td>
<td>ST</td>
<td>Persistence</td>
</tr>
<tr>
<td>5</td>
<td>Open Fracture of Tibia + OS</td>
<td>-</td>
<td>E. faecalis</td>
<td>Debridement &amp; OS removal</td>
<td>44 d</td>
<td>22 d</td>
<td>None</td>
<td>21 mo</td>
<td>Cured/Supersinfection</td>
</tr>
<tr>
<td>6</td>
<td>Femur OS</td>
<td>-</td>
<td>E. faecalis</td>
<td>Exchange (2 stage)</td>
<td>30 d</td>
<td>30 d</td>
<td>AMX 25 d</td>
<td>36 mo</td>
<td>Cured</td>
</tr>
<tr>
<td>7</td>
<td>Spine OS + Meningitis</td>
<td>+</td>
<td>E. faecalis</td>
<td>E. coli</td>
<td>Debridement</td>
<td>40 d</td>
<td>34 d</td>
<td>13 mo</td>
<td>Cured</td>
</tr>
<tr>
<td>8</td>
<td>Spine OS</td>
<td>-</td>
<td>E. faecalis</td>
<td>P. mirabilis</td>
<td>Debridement</td>
<td>7 d</td>
<td>7 d</td>
<td>AMX + CIP 32d</td>
<td>12 mo</td>
</tr>
<tr>
<td>9</td>
<td>Spine OS + Meningitis</td>
<td>-</td>
<td>E. faecalis</td>
<td>E. coli</td>
<td>Debridement</td>
<td>35 d</td>
<td>15 d</td>
<td>ACL 20d</td>
<td>40 mo</td>
</tr>
<tr>
<td>10</td>
<td>Chronic OM of the foot</td>
<td>-</td>
<td>E. faecalis</td>
<td>E. coli</td>
<td>Debridement</td>
<td>38 d</td>
<td>35 d</td>
<td>AMX + CXM 20d</td>
<td>19 mo</td>
</tr>
<tr>
<td>11</td>
<td>Endocarditis</td>
<td>+</td>
<td>E. faecalis</td>
<td>None</td>
<td>11 d</td>
<td>7 d</td>
<td>None</td>
<td>Death (hemorrhagic stroke)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1 - Summary of patients treated with Ampicillin-Ceftriaxone combination**

**Abbreviations:**
- ACL (amoxicillin-clavulanic acid)
- AMP-CRO (ampicillin-ceftriaxone combination)
- AMX (amoxicillin)
- CXM (cefuroxime-axetil)
- CIP (ciprofloxacin)
- d (days)
- IV (intravenous)
- mo (months)
- OM (osteomyelitis)
- OS (osteosynthesis)
- PJI (prosthetic joint infection)
- PO (oral)
- ST (suppressive therapy)
FIGURE LEGENDS

Figure 1
24 hour kill-curves with Ampicillin alone or in combination with Ceftriaxone against clinical strains of E. faecalis in exponential growth phase

Abbreviations: AMP (ampicillin), CRO (ceftriaxone), CFU (colony-forming units).
AMP-CRO combination results are shown for CRO 5 mg/l.

Case 3: AMP alone was bactericidal from 1 to 8 mg/l; higher concentrations were less active. AMP-CRO combination was synergistic for AMP concentrations of 0.12 to 0.5 mg/l, extending the bactericidal effect to AMP concentrations between 0.12 and 8 mg/l.

Case 4: AMP alone was bactericidal from 0.5 to 8 mg/l. AMP-CRO combination was synergistic for AMP concentrations of 0.12 to 0.25 mg/l, extending the bactericidal effect to AMP concentrations between 0.12 and 8 mg/l.

Figure 2
24 hour kill-curves with Ampicillin alone or in combination with Ceftriaxone against clinical strains of E. faecalis in stationary phase

Abbreviations: AMP (ampicillin), CRO (ceftriaxone), CFU (colony-forming units).
AMP-CRO combination results are represented for CRO 5 mg/l.

Cases 3 and 4: No bactericidal effect or synergy were observed.
Figure 1 – 24 h kill-curves with Ampicillin alone or in combination with Ceftriaxone against clinical strains of *E. faecalis* in exponential growth phase.
Figure 2 – 24 h kill-curves with Ampicillin alone or in combination with Ceftriaxone against clinical strains of *E. faecalis* in stationary phase

Case 3

<table>
<thead>
<tr>
<th>AMP concentration (mg/l)</th>
<th>log CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0.5 1 2 4 8 16 32 64</td>
<td>AMP AMP + CRO</td>
</tr>
</tbody>
</table>

Case 4

<table>
<thead>
<tr>
<th>AMP concentration (mg/l)</th>
<th>log CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0.5 1 2 4 8 16 32 64</td>
<td>AMP AMP + CRO</td>
</tr>
</tbody>
</table>