Introduction

Osteomyelitis is a term of Greek origin. In ancient Greek “osteon” and “myelos” means cortical bone and marrow respectively, and the ending “-itis” inflammation. Although relatively simple, this definition is not only a description of the disease, but also of its pathology.

Osteomyelitis is an inflammation of the bone and bone marrow caused usually by pyogenic bacteria, and rarely by mycobacteria or fungi (1). In adults the infection has mostly exogenous origin, following trauma or surgery; however, haematogenous spread can also happen. The end-stage of delayed or poorly treated acute osteomyelitis is the development of chronic infection, characterized by necrotic devascularised bone. Many predisposing factors have been described such as open fractures, malnutrition, alcoholism, malignancy, diabetes mellitus (DM), obesity, smoking, drug addiction, chronic steroid use and severe comorbidities (2, 3).

The incidence of Gram-negative multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacterial bone infections is increasing (4, 5). Emergence of resistance of pathogenic bacteria to multiple antimicrobial agents has become a significant public health issue. Literature lacks large series of gram negative bacterial bone infections, especially those caused by MDR, XDR or PDR organisms (4, 5).

A retrospective study was performed, aiming to investigate the causative organisms and to evaluate
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the proper surgical, as well as medical management and outcome of these infections.

**Patients and methods**

Between January 2007 and December 2016, all patients diagnosed with acute osteomyelitis caused by a gram negative MDR, XDR or PDR pathogen, treated in the University hospital of Heraklion, Crete, Greece, were included in the study. Patients were identified through a positive bone culture, from the hospital’s microbiological and medical records. The infection’s microbiological cause, its treatment and outcome were retrospectively evaluated. The patients’ demographics, possible predisposing factors, their score according to the American Society of Anesthesiologists (ASA) and their Charlson Comorbidity index were also evaluated. Prosthetic joint infections were excluded from this study.

Resistance was defined according to the criteria already described by Margiorakos et al. (6). MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories; XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, while PDR as non-susceptibility to all agents in all antimicrobial categories (6).

Three types of infection’s dissemination were recognized. Infections following internal fixation procedures were considered postoperative; haematogenous were those following another (primary) infection from the same pathogen and finally severe trauma related were those following an open fracture.

Eradication of the infection was defined as complete and prolonged disappearance of signs and symptoms of the disease, on the basis of negative cultures and a minimum of a 6-month follow-up. Persistence was defined as the continuation of signs and symptoms of the disease, confirmed by a positive culture.

The present study has been approved by the hospital’s bioethical committee.

The University hospital of Heraklion is a 700-bed, tertiary care and reference hospital for the whole island of Crete. The island’s population (approximately 700,000) is mixed rural, urban and suburban.

**Results**

A total of 14 osteomyelitis cases (6 females; 8 males) caused by a MDR or XDR Gram-negative pathogen were identified during the study period. Thirteen patients were adults, while there was also one pediatric case. Their mean age was 50.6 years (range: 10-76). The mean admission ASA score was 2.1, while the mean Charlson comorbidity index was 2.7. A total of 5 out of 14 patients were heavy and active smokers, 6 had suffered a severe injury, 4 were diagnosed with DM, 2 were obese and 2 had chronic renal failure. Additionally, 6 of them had received, in the last 3 months, antimicrobial treatment, while one was on chronic corticosteroid treatment due to rheumatoid arthritis.

Regarding the site of infection, 5 cases were of the femur [36%; 2 proximal, 2 shaft, 1 distal femur], 3 of the spine (2 lumbar; 1 thoracic spine) and 3 of the foot (21% each), followed by 2 cases of the proximal tibia (14%) and 1 of the pelvic bone (7%). The infection was postoperative (following an internal fixation procedure) in most of cases (8; 57%), with the mean duration between operation and development of infection being 7 days. In 4 (29%) was due to trauma (open fractures), caused by traffic accident, while in 2 cases (14 %) the spread has been considered haematogenous, with portal of entry the genitourinary tract.

A total of 16 MDR or XDR gramm negative microorganisms were isolated from bone cultures of the 14 study patients. PDR pathogens were not isolated. A total of 5 cases of *Acinetobacter baumanii* were recorded, with 3 of them being XDR and the remaining 2 MDR. Furthermore, 3 MDR *Klebsiella pneumoniae*, 3 MDR *Enterobacter cloacae* and 2 MDR *Escherichia coli* were isolated, while 2 isolates of *Pseudomonas aeruginosa*, 1 XDR and 1 MDR, were identified. A single case of a MDR *Roseomonas gilardii* was also identified.

Blood cultures yielded the same microorganisms as bone in 5 patients (36%): one case yielded MDR *Enterobacter cloacae*; a second MDR *Escherichia coli* with a MDR *Acinetobacter baumanii*; a third XDR *Acinetobacter baumanii* with an XDR *Enterococcus faecalis*; a fourth XDR *Acinetobacter baumanii* with a XDR *Pseudomonas aeruginosa* and a fifth MDR *Enterobacter cloacae* with a sensitive *Pseudomonas aeruginosa* (Table 1).

All 14 patients received first line empirical com-
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Thirteen patients were also surgically treated. Surgical intervention consisted in all cases of radical and in some cases repeated debridement (ranging from 1 to 4 times). Debridement was followed by removal of internal fixation in 2 cases and application of external fixation in 1.

The mean follow-up after discharge was 7.3 months. The microbiological outcome was eradication in 12 cases and persistence in 2, while the outcome was successful in 12, failure in 1 and death due to comorbidities in another. Two cases were treated in the Intensive Care Unit (ICU). The mean duration of hospitalization was 44.9 days.

### Table 1 - Demographics, Pathogens, Resistance, Site and Type of Infection Spread. A and B Pathogens Grew Together from Culture.

<table>
<thead>
<tr>
<th>N</th>
<th>Gender</th>
<th>Age</th>
<th>Pathogen A/Resistance</th>
<th>Pathogen B/Resistance</th>
<th>Pathogen-Blood culture</th>
<th>Site of infection</th>
<th>Spread type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>67</td>
<td><em>Escherichia coli</em> - MDR</td>
<td><em>Achromobacter xylooxidans</em>-Sensitive</td>
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<td>Foot</td>
<td>Trauma</td>
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<td>2</td>
<td>Male</td>
<td>44</td>
<td><em>Escherichia coli</em> - MDR</td>
<td><em>Acinetobacter baumanii</em>-MDR</td>
<td><em>Escherichia coli</em>- MDR/ <em>Acinetobacter baumanii</em>-MDR</td>
<td>Foot</td>
<td>Haematogenous</td>
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<td>3</td>
<td>Male</td>
<td>73</td>
<td><em>Enterobacter cloaca</em>- MDR</td>
<td>-</td>
<td>-</td>
<td>Foot</td>
<td>Postoperative</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>48</td>
<td><em>Enterobacter cloaca</em>- MDR</td>
<td><em>Staphylococcus epidermidis</em>-Methicillin resistant</td>
<td><em>Enterobacter cloaca</em>-MDR</td>
<td>Distal Femur</td>
<td>Postoperative</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>48</td>
<td><em>Pseudomonas aeruginosa</em>-MDR</td>
<td>-</td>
<td>-</td>
<td>Proximal tibia</td>
<td>Postoperative</td>
</tr>
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<td>6</td>
<td>Male</td>
<td>39</td>
<td><em>Enterobacter cloaca</em>- MDR</td>
<td><em>Pseudomonas aeruginosa</em>-sensitive</td>
<td><em>Enterobacter cloaca</em>-MDR/ <em>Pseudomonas aeruginosa</em>-sensitive</td>
<td>Proximal tibia</td>
<td>Postoperative</td>
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<td>7</td>
<td>Female</td>
<td>40</td>
<td><em>Acinetobacter baumanii</em>-XDR</td>
<td><em>Enterococcus faecalis</em>-Sensitive</td>
<td>-</td>
<td>Femur shaft</td>
<td>Trauma</td>
</tr>
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<td>8</td>
<td>Male</td>
<td>21</td>
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<td><em>Enterobacter cloaca</em>-Sensitive</td>
<td>-</td>
<td>Femur shaft</td>
<td>Trauma</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>76</td>
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<td><em>Pseudomonas aeruginosa</em>-XDR</td>
<td><em>Acinetobacter baumanii</em>-XDR/ <em>Pseudomonas aeruginosa</em>-XDR</td>
<td>Proximal femur</td>
<td>Postoperative</td>
</tr>
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<td>10</td>
<td>Female</td>
<td>75</td>
<td><em>Klebsiella pneumoniae</em>-MDR</td>
<td>-</td>
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<td>Haematogenous</td>
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<td>Female</td>
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<td>Pelvis</td>
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<td><em>Roseomonas gilardi</em>- MDR</td>
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<td>Postoperative</td>
</tr>
<tr>
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<td>Postoperative</td>
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<td>Spine</td>
<td>Postoperative</td>
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<td>Empiric treatment</td>
<td>Causative treatment</td>
<td>Appropriate empiric treatment</td>
<td>Microbiological outcome</td>
<td>Clinical outcome</td>
<td>Follow-up (months)</td>
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<tr>
<td>----</td>
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</tr>
<tr>
<td>1</td>
<td>Debridement x2</td>
<td>Ciprofloxacin/Rifampicin</td>
<td>Colistin/Amikacin/Imipenem/ Vancomycin</td>
<td>No</td>
<td>Eradication</td>
<td>Success</td>
<td>6</td>
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<tr>
<td>2</td>
<td>Debridement x4</td>
<td>Colistin/Tigecycline</td>
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<td></td>
<td>No Eradication</td>
<td>Failure</td>
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<tr>
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<td>Debridement, Removal of internal fixation</td>
<td>Ampicillin-Sulbactam/ Gentamycin</td>
<td>Ciprofloxacin/ Daptomycin/ Ertapenem</td>
<td>Yeas</td>
<td>Persistence</td>
<td>Failure</td>
<td>6</td>
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<tr>
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<td>Ciprofloxacin/Teicoplanin</td>
<td>Meropenem/Daptomycin/ Rifampicin</td>
<td>No</td>
<td>Eradication</td>
<td>Success</td>
<td>7</td>
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<td>Ciprofloxacin/Clindamycin</td>
<td>Piperacillin-Tazobactam/ Vancomycin</td>
<td>No</td>
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<td>Success</td>
<td>6</td>
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<tr>
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<td>Success</td>
<td>13</td>
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<tr>
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<td>Cefuroxime/Amikacin/Metronidazole</td>
<td>Meropenem/Colistin/Linezolid</td>
<td>No</td>
<td>Eradication</td>
<td>Success</td>
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<tr>
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<td>Tigecycline/Piperacillin-Tazobactam/Metronidazole</td>
<td>Gentamycin/Colistin/Tigecycline</td>
<td>No</td>
<td>Eradication</td>
<td>Success</td>
<td>6</td>
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<tr>
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<td>Debridement x3</td>
<td>Meropenem/Linezolid</td>
<td>Piperacillin-Tazobactam/Colistin/Tigecycline</td>
<td>No</td>
<td>Persistence</td>
<td>Death*</td>
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<td>No</td>
<td>Eradication</td>
<td>Success</td>
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<tr>
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<td>Eradication</td>
<td>9</td>
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<tr>
<td>12</td>
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<td>Yes</td>
<td>Eradication</td>
<td>8</td>
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<tr>
<td>13</td>
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<td>No</td>
<td>Eradication</td>
<td>Success</td>
<td>7</td>
</tr>
<tr>
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<td>Debridement x3</td>
<td>Tigecycline/Rifampicin</td>
<td></td>
<td></td>
<td>Yes</td>
<td>Eradication</td>
<td>6</td>
</tr>
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</table>

* Death due to comorbidities.
Discussion

Osteomyelitis can affect any bone and can be divided into three types: acute, subacute and chronic (7). The pathogens responsible are usually Gram-positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and coagulase-negative *staphylococci* species. Osteomyelitis caused by gram-negative pathogens is considered rare (8, 9). The present study evaluated retrospectively cases of acute MDR and XDR gram-negative osteomyelitis.

Literature lacks large series of osteomyelitis cases, their cause, treatment and outcome. The published cases due to gram-negative pathogens are even less, especially when it comes to MDR (4, 10). Therefore, the present study could be considered one of the few, single-center series of gram-negative, MDR and XDR acute osteomyelitis.

The mean age of the present patients was 50.6, and, if we exclude the pediatric case, it rises to 53.7 years. The female/male ratio was 0.75. Some other studies report prevalence of older male patients (11, 12). Large series do not exist, in order to extract safe conclusions; however, a reasonable assumption regarding the present “younger” group, could be that 4 out of 14 (29%) presented patients suffered severe (open fractures) traumas, representing the underlying cause of the infection. Accidents usually affect younger adults. Greece and especially the island of Crete has a very high prevalence of traffic accidents usually involving young adults (13, 14).

Regarding the causative microorganisms the present study has shown a higher prevalence of *Acinetobacter baumanii*, as compared to other series. Factors facilitating contamination, transmissions and multidrug resistance of this organism include widespread use of cephalosporins, quinolones and suboptimal hand hygiene (15). Furthermore, ICUs in Greece are constantly colonized by MDR *Acinetobacter baumanii*, being a spreading-source all over the hospital, a phenomenon also observed during the last 10 years in the University hospital of Heraklion, where the study took place (16, 17). The incidence of other isolated microorganisms such as *Escherichia coli* and *Klebsiella pneumoniae* are consistent with preceding studies (5, 11, 18). PDR pathogens were not isolated, despite the fact that such organisms exist in the hospital’s environment (19).

Quinolones, by being effective against a broad spectrum of bacteria and having good penetration in the bone, are frequently used by clinicians as empirical treatment of bone and joint infections (20). The population of the present study represented patients with MDR gram-negative osteomyelitis, with the causative organisms exhibiting high level of resistance in this type of antimicrobials. On the other hand colistin, an old agent that is being again extensively used, seems to be effective.

All present patients received initial empirical combination antimicrobial treatment, which has been proven effective in 4. The remaining 8 patients had a second line combination antimicrobial treatment based on the microbiological identification and sensitivities of the causative pathogens. Infections due to these MDR pathogens are a new reality, their treatment is a challenge, as the optimal treatment, either as monotherapy or as combination therapy has not yet been established (21).

The majority of patients (13/14) was subjected to surgical treatment as well. Surgical radical debridement is of paramount importance for the eradication of such infections. In cases that instrumentation implants have been affected, the removal of this hardware should also be considered. Different surgical treatment options are available for osteomyelitis at any stage: radical debridement, bone fenestration, reaming, the Masquelet-technique, segmental resection with callus distraction, bone grafting and even amputation (22). In the present study all cases including fixation implants (postoperative infections) were acute (mean duration between initial surgery and infection onset was 7 days). Therefore, radical debridement was the surgical treatment of choice with the addition in some, however not in all cases, the removal of implants and the application of external fixation for stabilization of the fractured site.

It is of note that 8 out 14 patients (57%) had undergone surgery (internal fixation), while 4 out of 14 (29%) had suffered severe trauma (open fractures) that most probably lead to the infection. Regarding other possible predisposing factors, 5 out of 14 (36%) were heavy and active smokers, 4 out of 14 (29%) were suffering DM, 2 (14%) were obese, 2 (14%) had chronic renal failure, while 1 (7%) was on chronic corticosteroid treatment due to rheumatoid arthritis. Due to the small number of patients and the absence of a control group, we cannot come to statistically significant conclusions. However, it seems that all these conditions had predisposed to
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the development of the infections. Prior surgical invasions and open fractures are well-known risk factors for osteomyelitis (5, 7). The tobacco use, that has an impact on vessels and micro-circulation (23), has already been identified as a risk factor, but has not been excessively studied in the existing literature (11). Furthermore, DM depresses the immunity and is a causative factor for a plethora of infections (24). The death of one patient of the present group was due to severe comorbidities. However, osteomyelitis could have contributed to the fatal outcome. Our results are in compliance with previously published studies (2, 4, 10, 11).

In 5 out of the 14 cases (36%) the same pathogen was also isolated from blood. Although concurrent bacteremia in osteomyelitis has not been methodically studied and reported, it appears that the rates of bacteremia are lower in Gram-positive osteomyelitis, which, in any case, are more often than their Gram-negative counterpart (25).

Limitations of the present study are the relatively small number of patients, coming from a single center and the absence of a control group. However, taking into account the rarity of gram-negative osteomyelitis, the present information can be useful for the management of similar cases. The present study has shown that MDR Gram-negative osteomyelitis is a dangerous reality, probably due to the inappropriate overuse of antimicrobial agents. Antimicrobial regimens represent an important supplement of surgical treatment of acute MDR and XDR gram-negative osteomyelitis. Combination of surgical and medical treatment with special attention to the proper use of antimicrobial agents is crucial for the eradication of such a difficult to treat infection.

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Disclosure of interest
The Authors report no conflict of interest.

References