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Research Article

7 Years of Experience in Osteomyelitis Management in Caracas, Venezuela

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Abstract

Objectives: To describe 7 years of experience in osteomyelitis management, from microbiological, clinical, radiological and laboratory points of view.

Material and methods: Data from clinical records from private practice in a Vascular Reference Center (Angios) located in Caracas, Venezuela; were collected retrospective, in which patients who have been diagnostic of chronic osteomyelitis and received treatment with daptomycin alone or in combination for attack phase (with curative intention); with the primary objective to evaluate microbiological and clinical outcomes and secondary endpoints as safety and efficacy. Patients evaluated among August 2009 and August 2016 were analyzed.

Result: 90 patients (68 males/22 females) with chronic osteomyelitis diagnosis were included; aged between 31 and 96 years (average 62,8 years), age over 65 years in 64 patients-71%; with comorbidities as diabetes mellitus (DM), High blood pressure (HBP) & peripheral vascular disease (PVD). The most frequently bacteria isolated in bone culture were Staphylococcus aureus (82 isolates with 76 strains resistant to methicillin and 68 strains with biofilm-positive production), coagulase- negative staphylococci (4 isolates, all methicillin-resistant & biofilm producers) and Enterococcus faecalis (4 isolates sensitive to Ampicillin and biofilm-negative production). Daptomycin was our election choice at attack phase as Outpatient Parenteral Antimicrobial Therapy (OPAT), at doses among 6 to 12mg/kg/day, alone (22 patients) or combine with Rifampicin at 600mg/PO/OD (68 patients); with mean treatment duration of 6,5 weeks (52, 5 days) (4-12 weeks). For consolidation phase, we considered 4 regimens pathogen-specific, Linezolid + Rifampicin for MRSA biofilm-positive, Moxifloxacin + Rifampicin for MSSA biofilm-positive; Moxifloxacin alone for MRS biofilm-negative and Ampicillin for Enterococcus faecalis biofilm-negative; for 4 weeks of therapy. Daptomycin resistance was absent (MICs over 1µgr/mL) and a reverse Creep MIC was observed), nor resistance develop to Linezolid, Moxifloxacin, Ampicillin or Rifampicin during treatment, and nor MIC Creep phenomenon appears. The overall treatment success rate (clinical, microbiological, radiological and laboratory values normalization) was 100% (healing). Daptomycin regimens were used in 37 patients (41,1%) as a rescue medication, and only in 21 patients as first option choice (23,3%). 16 adverse events were documented, 10 as severe fatigue and 6 CPK elevations (under 5 times NL), which not forced discontinuation of the drug in any patient (further analysis showed no causality in any case), and no reports of nephrotoxicity, pneumo, muscle-toxicity, bone narrow suppression, peripheral neuropathy or liver abnormalities were observed.

Conclusion: In Venezuelan experience, chronic osteomyelitis produced by Gram-positive cocci with high antimicrobial resistance profile and biofilm expression, the cornerstone of attack phase was Daptomycin (alone or combined with Rifampicin), follow by a consolidation therapy with slow or faster bactericidal drugs (linezolid+/-rifampicin, moxifloxacin+/-rifampicin, ampicillin), with excellent efficacy, tolerance and safety profile at the OPAT setting even in elderly patients with severe comorbidities, with a great rate of clinical, microbiological, radiological and paraclinical cure.

Keywords: Osteomyelitis; Biofilm; Daptomycin; Linezolid; Moxifloxacin; Rifampicin; Gram-positive; OPAT; Safety; Efficacy; Venezuela; Diabetic

Introduction

In Venezuela, Gram-positive chronic osteomyelitis was a challenge for the physicians, due to the lack of tailor-made treatment protocols according to microbiology isolates susceptibility aligned with national or international therapeutic guidelines. In addition, elderly or immunosuppressed patients have a lot of comorbidities, that difficult the selection of the right antimicrobial therapy.

Landscape is more complicated when an additional pathogenic factor is present: the biofilm production, that turns the bacteria into a superbug, because generates resistance mechanism not only to antimicrobial drugs, also against environmental changes (temperature, rheostatic, humidity, nutrients depletion, etc.), with the final result of infection persistence.

Drugs with activity in bone infection were limits due to its minor concentration in bone tissue compared with its plasmatic concentration. Another factor that condition the use of drugs in bone infection is related to its cidality, quick-bactericidal drugs were preferred over slow-bactericidal drugs, because the opportunity to generate or express a resistant mechanism like mutation in a target enzyme, change in a transportation protein, expression of an efflux pump, etc., are more frequent and feasible is the attack to the bacteria is slow. Another aspect is the presence of post-antibiotic effect, that means, that antimicrobial action continues over the therapeutic target even the plasmatic (or tissue) concentration of the drug diminish, because the MoA (mechanism of action) of the drug implicates an irreversible union to the therapeutic target. A key factor to drive the selection of a drug for bone infection is its anti-biofilm activity, because it can determine treatment duration, drugs to combine, toxicity, and clinical and microbiological expected outcome. A brief overview of the drugs available in Venezuela for chronic osteomyelitis treatment is presented next.

Daptomycin (Cubicin®), the first-in-class cyclic lipopeptide antibiotic, was approved in Europe for the treatment of Complicated Skin and Soft Tissue Infections (cSSTIs) in 2006 and for the treatment of right-sided infective endocarditis (RIE) due to *Staphylococcus aureus* and *S. aureus bacteremia* (SAB) when associated with RIE or with cSSTI in 2007 [1]. It was available in Venezuela since 2009, and represents an opportunity to improve severe Gram-positive infections treatment. Daptomycin is bactericidal against Gram-positive bacteria, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Vancomycin-Resistant Enterococci* (VRE), it kills Gram-positive bacteria by a novel mechanism of disruption of multiple bacterial plasma membrane functions, without penetrating the cytoplasm [2]. Insertion of the lipophilic daptomycin tail into the bacterial cell membrane with oligomerization and channel formation causes rapid membrane depolarization and a potassium ion efflux. Arrest of DNA, RNA, toxin production, and protein synthesis follows, resulting in bacterial cell death without lysis of the cell wall, which gives a further advantage in diseases where inflammatory response associated counter antimicrobial use producing cell lysis [3-5].

The in vitro potency of daptomycin has been demonstrated against a wide range of aerobic and anaerobic Gram-positive bacteria, including MRSA, glycopeptide-intermediate *S. aureus* (GISA) *vancomycin-resistant S. aureus* (VRSA), *methicillin-resistant coagulase-negative staphylococci* (MRCNS), and VRE. Synergy with daptomycin has been described in vitro with aminoglycosides, i.e., gentamicin, oxacillin, other b-lactams, macrolides and rifampicin. Daptomycin exhibits a dose-dependent post-antibiotic effect lasting from 1 to 6 hours against *E. faecalis* and *S. aureus* after exposure to concentrations ranging from 0.25 to 16mg/L (i.e., between one- and eightfold the MIC) [2,6-8].

Pharmacology of daptomycin is interesting, with an 8-hours half-life, once-daily dosing results in linear pharmacokinetics at doses up to 12mg/kg, with minimal drug accumulation. Daptomycin distributes primarily in the plasma, with penetration to vascularized tissues. The drug is highly protein-bound (92%); excretion occurs primarily via the kidneys. Approximately 80% of the total dose, of which two-thirds is intact drug, is recovered in the urine. In patients with severe renal impairment (creatinine clearance < 30mL/min), the dosing interval is increased from once daily to every 48 hours. Daptomycin's unique mechanism of action and its lack of metabolizing by cytochrome p450 or other hepatic enzymes results in an absence of drug–drug interactions. Synergistic interactions were observed between daptomycin and gentamicin against *staphylococci* and *enterococci*, including strains resistant to methicillin and vancomycin (valuable to biofilm disease infections treatment); in vitro synergistic interactions of daptomycin and rifampin have been shown against MRSA, VRSA and VRE, with a great anti-biofilm activity and reduction of the rifampicin resistance appearance. Not antagonism interactions were observed with daptomycin use in combination with several antimicrobial agents, only additive, synergistic effect or indifference were reported.

Skeletal muscle was the most sensitive tissue to the adverse effects of daptomycin in animal studies. Mild myopathy was easily predicted and monitored by measuring serum creatine phosphokinase concentrations; the effect was reversible upon the cessation of therapy [9].

Rifampicin, a semisynthetic antibiotic produced from *Streptomyces mediterranei* has a broad antibacterial spectrum, including activity against several forms of *Mycobacterium sp.* and is the only antimicrobial with high activity against biofilm producer organism. Rifampin acts via the inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death, but its MoA over biological films was not completely clear [10]. The higher rate of resistance to the drug in single regimen was the reason to left out of the single-drug schemas in biofilm diseases [11]. Its low gastric tolerance (but great oral bioavailability) and liver toxicity limits its use in combination therapy. Another fact against its use was is great potential of drug-drug interactions due mainly to its hepatic metabolism and high protein binding [12].

In patients with high-risk of limb amputation due to bone infections, despite rifampicin negative considerations, was a great drug in combinations, with a risk/benefit ratio affordable with good monitoring [13]. Rifampicin price is almost inexpensive compared with its pairs in combination (daptomycin, linezolid or moxifloxacin). Our previous favorable experience in combination therapy in vertebral osteomyelitis due to Mycobacterium tuberculosis (Pott's disease), give us confidence in its potential against biofilm disease in other infected bones.

Linezolid is an oxazolidinone, discovered by DuPont Pharmaceuticals in the late 1980s but linezolid, the first analogue suitable for development, was found only when the family was re-examined by Pharmacia in the 1990s. Oxazolidinones bind to the 50S subunit of the prokaryotic ribosome, preventing formation of the initiation complex for protein synthesis. This is a novel mode of action; other protein synthesis inhibitors either block polypeptide extension or cause misreading of mRNA. Linezolid MICs vary slightly

with the test method, laboratory, and significance attributed to thin hazes of bacterial survival, but all workers find that the susceptibility distributions are narrow and unimodal, with MIC values between 0.5 and 4mg/L for *streptococci*, *enterococci* and *staphylococci*. Full activity is retained against *Gram-positive cocci* resistant to other antibiotics, including *methicillin-resistant staphylococci* and *vancomycin-resistant enterococci*. MICs are 4-8mg/L for Moraxella, Pasteurella and *Bacteroides spp*. but other Gram-negative bacteria are resistant as a result of endogenous efflux activity. Resistance is difficult to select in vitro but has been reported during therapy in a few enterococcal infections and in two MRSA cases to date; the mechanism entails mutation of the 23S rRNA that forms the binding site for linezolid. Risk factors for selection of resistance include indwelling devices, undrained foci, protracted therapy and underdosage [14]. Actually, Linezolid MoA define is cidal activity as a slow-bactericidal in place of a bacteriostatic previously named, and that is one of the reason that puts the drug in the consolidation or suppressive phase of osteomyelitis treatment.

Effectiveness and safety of linezolid administration for the treatment of patients with orthopedic infections due to multidrug-resistant Gram-positive cocci is widely known right now, it needs a narrow clinical and laboratory control to detect the adverse effects in prolonged administration (i.e. osteomyelitis) especially bone marrow suppression with thrombocytopenia & anaemia as key points, and linezolid-induced peripheral neuropathy that leads to discontinuation because this may be an irreversible event [15].

Despite linezolid rapidly reaches infected tissue compartments of joints and the tissues surrounding bone in concentrations greater than twice the MIC for 90%, however, intra-bone tissue concentrations of linezolid below the MIC for 90% also occur; thus, an aggressive surgical approach to bone infection should be taken if possible [16]. That's the key success factor in our center, the multidisciplinary approach to the patient, where the surgeons' participation with a reduction of the infective biomass leads to a better concentration of the drugs, minimize biofilm neutralization & protective factors and could be determine a shortage of the treatment duration.

Moxifloxacin is one of the most powerful quinolones, with the best and faster bactericidal action, broad spectrum (including anaerobic bacteria), great oral bioavailability and tolerance [17]. Great bone penetration (and concentration), with an excellent post-antibiotic effect and favourable PK/PD parameters that leads to an oral administration once daily, that improves gastric tolerance over ciprofloxacin and even over levofloxacin (oral once-daily administration) [18-20].

Development of resistance under a course of treatment is less frequently than with other quinolones, but, MRSA is one of the bacteria that changes its molecular targets to avoid moxifloxacin bactericidal action [21]. In the Microbiology laboratory, we have the opportunity to test consecutive bone samples of diabetic patients that received treatment with moxifloxacin as monotherapy for chronic osteomyelitis, isolates have a demonstrated susceptibility according to initial *in vitro* test, but, with clinical failure was present in the evolution of the patient, so, when we performed the identification procedure for a new bone sample, a surprise was coming, the same initial strain still viable, and now express a phenotypic biofilm production pattern, and when the susceptibility test was performed, another surprise, moxifloxacin resistance is now present. The analyze of these behavior pattern against moxifloxacin (especially with MRSA isolates), lead us to avoid moxifloxacin use as monotherapy for the attack phase in the chronic osteomyelitis treatment, with special emphasis in the diabetic foot osteomyelitis, and we decided to reserve its use for a consolidation phase (as monotherapy is biofilm production is negative) or in combination if the strain isolated is a biofilm-producer.

Ampicillin was a betalactamic drug reserved for enterococci bone infection, because susceptibility to this drugs stills remains good and bone penetration and concentration achieve cidality standards [22]. By general rule, Enterococci are not biofilm-producer, an its MICs for Ampicillin remains low with a good susceptibility especially for Enterococcus faecalis, that's the most common enterococci isolated in bone samples. One characteristic of E. faecalis is the absence of biofilm production in bone infection, that helps physicians in their antimicrobial management [23].

Ampicillin has a medium bioavailability (30-40%), poor protein bound (15-25%), urinary excretion over 90%, and bone penetration nears 20% of plasmatic concentration, and a good tolerance, except for diarrhoea as its major adverse event, still as a good option for enterococci chronic osteomyelitis. The price is cheaper and availability of ampicillin in Venezuela is good even in the last two years [24].

As a national reference center for patients with chronic wounds in limbs, especially legs, Angios Vascular Center and Wound Clinic specializes in a multidisciplinary approach form different medical and surgical specialties to saving limbs at risk of amputation due to peripheral vascular disease (arterial and/or venous) diabetes mellitus, trauma, severe infections, etc. The antimicrobial use restrictions due to advanced age, renal and hepatic impairment, allergies; multidrug-resistant pathogens (because many patients comes from healthcare centers or nursing houses), were part of a complicated landscape for management of a chronic infectious disease as osteomyelitis.

In Venezuela, pharmacoeconomics concepts application leads to precognize ambulatory therapy (even parenteral) as a reduction of monetary costs and risk-reduction measure (to avoid colonization or infection for multidrug-resistant pathogens during a prolonged hospitalization), so, we prefer the use of outpatient parenteral antimicrobial therapy (OPAT) for the management of chronic osteomyelitis.

Part of the information of this manuscript comes from one author participation as main investigator of the EUCORE(SM) study (European Cubicin® Outcomes Registry and Experience), a multicenter, retrospective, non-interventional registry sponsored by Novartis Pharma AG that collected data across 18 countries (Europe, Latin America & Asia). However, because of their rigorous

nature (e.g. inclusion and exclusion criteria and the requirement for protocol adherence), clinical trials may not always reflect the true experience with a drug in clinical practice, patient registries such as EUCORE(SM) provide insight into real-world clinical experience with daptomycin and build on the evidence base from clinical trials and "customized" for every country and for long-time periods in many pathologies [25,26].

Objectives

A. Describe 7 years of experience in osteomyelitis management, from microbiological, clinical, radiological and laboratory points of view.

B. Shows Daptomycin (alone) and its combinations efficacy in chronic osteomyelitis treatment.

C. Shows the benefits of OPAT for severe and long-term Gram-positive diseases.

D. Shows Daptomycin (alone) and its combinations safety profile for use in elderly patients with multiple comorbidities.

Material and Methods

Retrospective review of clinical records from patients evaluated and treated of Angios Vascular Center & Wound Clinic among August 2009 and August 2016 were analyzed. Patients included were diagnosed with chronic osteomyelitis and treated at Angios by a medical multidisciplinary team. Data were collected from clinical record was tabulated in spreadsheets were compose to organize information and promotes the analysis using comparative statistics (percentages) and summarized.

Result and Discussion

A total of 90 patients (males/22 females) were included; aged between 31 and 96 years old (average 62,8 years), age over 65 years in 64 patients (71%), so, this high number of elderly patients supposed an increased immunosuppression condition aged-related. Comorbidities were present in all patients, the most common was diabetes mellitus (DM) (31-34%), and its combinations with PVD (Peripheral Vascular Disease) + HBP (High Blood Pressure) (26), HBP (21) and PVD (3), with a total of diabetic patients of 81 (90%). Demographic information was summarized in Table 1.

Table 1: Demographics characteristics of chronic osteomyelitis patients. Medical Microbiology Unit, Angios Vascular Center, Caracas, Venezuela. August2009-August 2016.

Patients (number)	Age (years)	Age Media (years)	Sex	Comorbidities
90	31-96	62,8	68M/ 22F	DM=31
				DM+PVD+HBP=26
				DM+HBP=21
				DM+PVD=3
				PVD+HBP=4
				HBP=3
				PVD=2

DM: Diabetes Mellitus; PVD: Peripheral Vascular Disease; HBP: High Blood Pressure

Most of our patients (70) have non-insulin-dependent diabetes mellitus (type 2), in contrast with 11 patients with insulin-dependent diabetes mellitus (type 1), that´s a reason for later assistance to healthcare services in the infectious setting, because in patients with type 1 diabetes mellitus, the infection process leads to a severe metabolic decompensation (diabetic ketoacidosis or hyperosmolar coma), that needs urgent medical attention for its life-threating condition. In type 2 patients, the infection is more insidious over the metabolic control, it causes hyperglycemia, but the oral hypoglycemic agents times, so, the patients search for medical care is delayed, and infection damages are more severe when it comes for healthcare support.

The vascular damage of hyperglycemia leads to the PVD & HBP, that become in a part of a vicious circle, when the venous and/or arterial damage limits the availability of oxygen to the tissue, decrease antimicrobial drugs concentration &reduces immune response due to a minor activation of the pro-inflammatory mediators & cells transport. In addition, low oxygen levels with a favorable oxidation-reduction potential, are auspicious for bacterial infection and moreover, for biofilm expression for these bacterial agents, with the subsequent increase in antimicrobial resistance [27]. Type 2 diabetes predominates with 8 patients over 3 with Type 1 disease, it could be explained because these patients goes to healthcare service later than type 1 where a metabolic decompensation (diabetic ketoacidosis or hyperosmolar coma) comes associated with the infectious process, but Type 2 patients not necessarily present this severe metabolic decompensation related to the infection.

The combination of DM + PVD + HBP had the worst initial prognosis, but finally a satisfactory therapeutic response [27], but these patients require additional efforts, such as peripheral vascular catheterization and adequate blood pressure control, in addition to glycemic control Focus on the infective microorganisms, the most frequently isolated was *Staphylococcus aureus* (82-91, 1%-), and methicillin resistance was present in 76 isolates (97, 7%). Of the total of methicillin-resistant Staphylococcus aureus (MRSA), 60

isolates expressed biofilm production (79%). Of the 6 isolates of *S. aureus* methicillin-susceptible (MSSA), 4 were positive for biofilm production. These observations were according to the etiologic role of *S. aureus* in chronic osteomyelitis in general population & diabetic patients, but some authors consider that MRSA coverage is not the rule, just the exception, and biofilm production was not analyzed by many of them, so, the role of it in the pathogenesis of chronic osteomyelitis still waiting to be reveal [28].

All *S. aureus* strains lacking yellow-golden pigment production, these being associated with severe chronic and deep tissue infections, instead of surface infections caused by pigmented strains (e.g. uncomplicated cellulitis), being this feature phenotypic importance to the treating physicians when goes to the microbiology laboratory to solicitude a preliminary result of a bone culture, to suspect an aggressive bacteria and possible biofilm production.

Secondly in order of frequency, 4 strains of *Enterococcus faecalis* (all isolates sensitive to Ampicillin and biofilm-negative production) and 4 strains of coagulase-negative staphylococci -CNS- (all methicillin-resistant & biofilm producers). A resume is presented in Table 2.

Table 2: Characterization of microorganism causative of chronic osteomyelitis. Medical Microbiology Unit, Angios Vascular Center, Caracas,Venezuela. August 2009-August 2016

Microorganism Isolated from Bone Culture	Nr.	Biofilm Production	
		(+)	(-)
Methicillin-Resistant *S. aureus* (MRSA)	76	60	16
Methicillin-Susceptible *S. aureus* (MSSA)	6	4	2
*Enterococcus faecalis**	4	-	4
Methicillin-Resistant *S. epidermidis* (MRSE)	3	3	-
Methicillin-Resistant *S. haemolyticus* (MRSH)	1	1	-

*All isolates susceptible to Ampicillin.

One of the real-problems in chronic osteomyelitis is the antimicrobial resistance, an even more, in the biofilm production, that not only limited the antimicrobial action, it increases the embolic phenomenon with a distance spread of the infection (in the same bone and to others, i.e. in diabetic foot from the phalanges to the metatarsus and tarsus); and produces an increase in vascular deficiency due to the obstruction of the microvasculature, with increased tissue hypoxia.

A greatest problem of all those associated to the presence of microorganisms producers of biofilms is given by the impossibility of reaching adequate concentrations of antimicrobials to eliminate said biofilm, since the inactivation by alginate polymers, entrapment by excess of therapeutic targets within the biomass, efflux by pumping systems, modification of the metabolic activity of the bacteria with reduction of the activity of the antimicrobial that acts on processes of active synthesis; besides the guarantee of a reserve of nutrients by "cannibalism" phenomena within the biofilm; all this makes that treatment of chronic osteomyelitis caused by biofilm-producing bacteria is complicated [29].

In addition, the inflammatory and immunological response is affected by the acidophilic medium that is propitiated within the biofilm, which also contributes to the persistence and inactivation of the antimicrobial agents. And as if this were not enough, the biofilm masks antigenic determinants and provides a supreme adhesion to the epithelia where it develops. Moving to the antimicrobial management, in our center due to the possibility of perform a bone biopsy to obtain a representative sample for culture, we guide the antimicrobial selection based on microorganism characteristics (as biofilm production) & susceptibility test results.

The attack phase for those biofilm-producing microorganisms with resistance to methicillin lasted from 6 to 12 weeks, depending on the patient's clinical, laboratory and imaging evolution, in addition to evaluating the adverse events that occurred. The therapy of choice was the combination of Daptomycin at doses between 6 and 12mg/kg/day (given intravenous) combined with Rifampicin at doses of 600 mg PO OD, which was administered to 68 patients. In the 22 patients with isolates of non-biofilm producing microorganisms, the initial therapy was Daptomycin as mono drug, at the doses and times already mentioned.

In the consolidation (or suppression) phase, microorganisms isolates producing biofilms two therapeutic schemes were used, for the 64 patients with infection by microorganisms resistant to methicillin, the combination of Linezolid at doses of 600mg PO BID plus Rifampicin at doses of 600mg PO OD for 4 to 6 weeks was selected; in the case of susceptibility to methicillin the combination of Moxifloxacin at doses of 400mg PO OD and Rifampicin at the dose mentioned above was used in 4 patients for 4 weeks.

Also in the consolidation phase, monotherapy with Moxifloxacin at standard doses for 4 weeks was used in the 16 patients with methicillin-resistant but negative biofilm production

microorganisms. In the 4 patients with *Enterococcus faecalis* isolates (ampicillin susceptible and negative for biofilm production), monotherapy with Ampicillin at standard doses (500mg PO QID) was used for 4 weeks.

The presence of adverse events was clinically and para clinically monitored at least once a week, and in elderly patients or in those in which high doses of daptomycin were prescribed, the frequency increases until 2 or 3 times a week. Daptomycin was used as Outpatient Parenteral Antimicrobial Therapy (OPAT), in order to reduces costs and new infection risks for the patients, and to improve life quality during the treatment. The consolidation phase of the treatment only includes oral medication, but continues with clinical and laboratory controls according to the physician scheduled [25].

For Daptomycin OPAT, patients arrived at the medical center, to a special ambulatory treatment facility room, and received Daptomycin infusion for a 30 minutes time or the bolus injection in 2-5 minutes time [30] at approved doses of 6mg/Kg/day or >6mg/kg/day (unapproved dose, used based on anti-biofilm previous successful reported experiences) [31].

Table 3: Characterization of isolates, biofilm production and daptomycin dose & length for attack phase in patients with chronic osteomyelitis diagnosis. Medical Microbiology Unit, Angios Vascular Center, Caracas, Venezuela. August 2009-August 2016.

	Daptomycin Dose (mg/Kg)				
Microorganism, Biofilm Production & Treatment Length	6	8	10	12	Total overall
MRSA	15	27	23	11	76
‡ Biofilm-Production Positive	3	25	22	10	60
4 weeks		1	5	3	9
6 weeks	1	14	8	6	29
8 weeks	2	9	6	1	18
12 weeks		1	3		4
Biofilm-Production Negative	12	2	1	1	16
4 weeks	4	1	1	1	7
6 weeks	8	1			9
MSSA	1	4	1		6
‡ Biofilm-Production Positive		3	1		4
6 weeks		2			2
8 weeks		1			1
12 weeks			1		1
Biofilm-Production Negative	1	1			2
4 weeks	1	1			2
Total overall	16	31	24	11	82

MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Susceptible *Staphylococcus aureus*

Daptomycin regimens were used in 21 patients as first option choice (23, 3%), in 32 patients (35, 6%) as second drug after a first treatment failure, and as a rescue medication (three or more drugs previously used) in 37 patients (41, 1%). The use of daptomycin as monotherapy in patients with isolates negative for biofilm production, implicates a minor treatment length and a minor drug dosage, without compromising the final outcomes. In previous observations daptomycin monotherapy achieves a complete cure in a diabetic patient infected with an isolate of MRSA positive for biofilm production [32] (Table 4).

The overall treatment success rate was 100% (complete healing), that means that 4 criteria were fulfilled:

A. Clinical cure: determinate by fistula & wound (ulcer) healing with pain reduction.

B. Laboratory (Paraclinical) cure: reduction or normalization of Erythrocyte Sedimentation Rate (ESR), C Reactive-Protein (CRP) & alkaline phosphatase (AP) values.

C. Imagenologic cure: defined by nor new osteolytic/

osteoblastic signs apparition, nor fractures; signs of osteosynthesis, or, three-phase bone scintigraphy with ciprofloxacin negative at the end of treatment (that continues negative in the next 3, 6 or 12 months later).

D. Microbiological cure: was determined by negative bone culture at the end of the attack phase or start the consolidation phase (first week).

Table 4: Characteristics of treatment regimens for consolidation phase in patients with chronic osteomyelitis diagnosis. Medical Microbiology Unit, Angios Vascular Center, Caracas, Venezuela. August 2009-August 2016.

Microorganisms & Biofilm Production	Treatment Regimens & Length (weeks)				Total Overall
	LZD + RD	MOX	MOX + RD		
	4	6	4	4	
Positive	54	10		4	68
MRSA	50	10			60
MRSE	3				3
MRSH	1				1
MSSA				4	4
Negative			18		22
MRSA			16		16
MSSA			2		2
Total Overall	54	10	18	4	90

LZD: Linezolid (600mg PO BID); RD: Rifampicin (600mg PO OD); MOX: Moxifloxacin (400mg PO OD); MRSA: Methicillin-Resistant *Staphylococcus aureus*; MRSE: Methicillin-Resistant *Staphylococcus epidermidis*; MRSH: Methicillin-Resistant *Staphylococcus haemolyticus*; MSSA: Methicillin-Susceptible *Staphylococcus aureus*; Data not showed: *Enterococcus faecalis* 4 isolates Biofilm-producer Negative. Treated with Ampicillin for 4 weeks.

The follow-up of these patients includes a visit every 3 or 6 months during first year after finish of treatment, and then every year (or sooner if is necessary). A clinical exam, review of laboratory tests and radiologic images were performed, without evidence of reactivation of the initial eradicated bone infection. Daptomycin regimens independently of its use (first of second choice, or as rescue treatment) shows great efficacy. [27,33] Safety of Daptomycin containing-treatments were demonstrated by the pharmacovigilance findings of 16 adverse events (AE). 10 cases of severe fatigue (asthenia) were identified, 8 in patients that received daptomycin + rifampicin treatment, nor related to the dose or treatment length. The other AE reported was CPK elevation (under 5 times normal levels), all of it in patients whom received daptomycin + rifampicin, 4 patients received 10mg/Kg dose for 12 weeks, 1 patient received 8mg/Kg dose for 12 weeks and 1 patient received a 10mg/Kg dose for 4 weeks; it shows that higher doses (10mg/kg) a prolonged treatment time (12 weeks), increase the possibility of the appearance of this adverse event [34-39].

All adverse events were documented after a minimum of 4 weeks of treatment. None of the 16 patients had rhabdomyolysis or severe myalgias leading to discontinuation of treatment. Interestingly, these patients received regimens with rifampicin again in their consolidation treatment phase, without presenting adverse events, which suggests that the combination of daptomycin + rifampicin may favor the onset of these adverse events.

The management of this AE leads to a closer daily monitoring of creatine phosphokinase (CPK) values, increase parenteral hydration, but not needs a dose reduction, because the patients were strictly follow in order to maintain the bacterial eradication benefit of the antimicrobial agent use. All the patients who received statins they should discontinue use during antimicrobial therapy, to reduce risk of muscle toxicity. No reports of pneumo, hepatic, neurologic or nephrotoxicity were documented, in a similar way to the literature reports [25-27].

A paradoxical finding was observed in relation to the reports of antimicrobial susceptibility to Daptomycin when comparing isolates from patients in the first 3 years of observation (2009-2012) and the remaining 4 years. Daptomycin resistance was absent, with Minimal Inhibitory Concentrations (MICs) below 1µgr/mL cut-off, so, the absence of resistance could be explaining because Daptomycin is brand new antimicrobial agent, and bacterial isolates never facing, and lacks of cross-over resistance mechanism due to its unique mechanism of action in the bacterial plasmatic layer. So, a MIC Creep phenomenon was not observed in this 3-year period (there was no increase in MIC values within the range of susceptibility). But, the paradox comes with an unexpected observation, and phenomenon called "Reverse MIC Creep", expressed by reduction of the MIC values inside the "susceptible" range, and it was observed between the first three years, when initial MICs values were over 0.125µgr/mL, compared with the last four years, when it reduces to a mean of 0.064µgr/mL. Maybe it could be explaining by 3 factors, the novel MoA of the drug, high cidality activity (rapid bactericidal), and anti-biofilm action, leading all of them to the growth in its use.

Conclusion

The possibility to cure chronic osteomyelitis even in patients with severe comorbidities is real, it needs a multidisciplinary medical team, determine the causative agent, the use of bactericidal

and anti-biofilm antimicrobial agents for prolonged time with a strictly medical follow-up. OPAT with daptomycin at higher doses+ rifampicin was an excellent combination against biofilm-producer MRSA, with great efficacy and safety profile, well tolerated in elderly patients with multiple comorbidities, leading to a complete eradication of the bone infection. Consolidation therapy for a short-period using quick + slow bactericidal drugs with anti-biofilm activity helps to maintain the results achieving in the attack phase.

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