A puzzling microbiological and clinical discrepancy in the management of acute, severe skin-soft tissue and joint staphylococcal infection. 

In vitro antimicrobial susceptibility to glycopeptides, versus in vivo clinical efficacy of linezolid alone

INTRODUCTION

Gram-positive infections represent an emerging threat for clinicians and microbiologists, due to their increasing frequency in both hospital and community setting, and the significantly growing rate of methicillin-resistant and multiresistant gram-positive cocci, predominantly staphylococci, enterococci, and streptococci [1]. The rate of methicillin-resistance of staphylococci may be greater than 60-70% in some selected hospital wards (i.e. intensive care units, but also Pneumology departments) [1, 2], and the report of glycopeptide-intermediate and glycopeptide-resistant strains is also on increase, therefore leaving very poor pharmaceutical tools for these severe infectious complications, although some “ol-
ment of these emerging multiresistant gram-positive cocci [1-6].

Linezolid is the first oxazolidinone antibiotic, available for both i.v. and oral administration, which thanks to a unique mechanism of action, retains activity against strains of gram-positive cocci who became resistant to methicillin and glycopeptides, too [1, 5, 7]. The cumulated clinical experiences in different clinical settings may confirm its favourable efficacy and tolerability features in a broad spectrum of possible indications, including respiratory tract infection, skin and soft tissue infection, infected diabetic foot, septicemia, endocarditis, central nervous system disorders, as well as bone and joint infections, and orthopedic and prosthetic surgery where the incidence of multiresistant staphylococcal infection is of particular concern [5, 7-20].

The elevated distribution volume and the low protein binding allow an enhanced tissue penetration of linezolid, with four-fold greater drug concentrations retrieved in alveolar fluids, and a 80-90% penetration into muscle, bone, and sinovial membranes, and 60% into bone, compared with plasma concentrations [5]. On the other hand, bone concentrations of teicoplanin and vancomycin reach 50-60% and around 10% respectively (when compared with plasma levels) [3, 21], therefore suggesting favorable clinical indications of linezolid in bone and joint infection, as well as local subcutaneous tissues. The demonstrated tolerability and safety of linezolid makes possible the administration of this novel antibiotic also to adolescents, children, and neonates, suffering from confirmed, resistant gram-positive cocci infections [7, 12, 22].

Also from a pharmacoeconomic point of view, the intrinsic elevated cost of linezolid is balanced by the availability of an oral formulation allowing a rapid switch to oral route of administration, and may be effectively counterbalanced by a reduced admission time [9, 12, 23, 24]. In a multicentre trial of linezolid versus teicoplanin carried out in 227 patients with serious and multiresistant gram-positive infections, a shorter length of admission and lower mean total costs of treatment were demonstrated for the linezolid versus the teicoplanin group [24]. The possibility to resort to the oral linezolid formulation (which maintains a 100% bioavailability compared with i.v. administration), allows reduced hospitalization and an effective outpatient management of infections which need prolonged treatment courses, just like bone and joint infections [5, 7]. Actually, the major cost of treatment of bone and joint infections is primarily driven by the long therapeutic courses, and especially the length of hospital stay. A retrospective audit of 55 episodes of orthopaedic infections with teicoplanin delivered in the outpatient or home setting significantly reduced associated expenditures compared with the ambulatory setting, and especially an hypothetical continued inpatient treatment. More extensive advantages are expected with the i.v. formulation and oral-bioavailable linezolid [25]. The indicators of quality of life advantages from both the hospital’s and subject’s perspectives are also expected to be improved by such a therapeutic choice [25]. Finally, although acquired linezolid resistance has been already reported and is also expected to increase over time [24], its growing frequency may counterbalance the elevated selective pressure exerted until now by the widespread use of glycopeptides on both patient and hospital flora, and indirectly contain the rising emergence of glycopeptide-resistant strains.

A puzzling case report of a septic post-surgical staphylococcal arthritis not responsive to long-term courses of associated rifampicin and teicoplanin or vancomycin despite apparently favourable in vitro susceptibility assays, but rapidly resolved by i.v. followed by oral administration of linezolid is presented and discussed on the ground of the most recent literature evidences.

### CASE REPORT

A 45-year-old man with HIV infection diagnosed since ten years, was successfully treated since eight years with different antiretroviral therapeutic lines, with the last combination including lamivudine, tenofovir, and nelfinavir. On admission, his CD4+ lymphocyte count was 365 cells/µL, his viral load was undetectable (<50 HIV-RNA copies/mL, by the ultrasensitive branched-DNA assay), and HIV disease remained clinically mute since early diagnosis. An incidental, closed trauma of the left knee, complicated by a polyfragmentary fracture of the homolateral patella, in absence of any exposed lesion, led to a scheduled orthopaedic intervention of left hemipatellectomy which did not require antimicrobial chemoprophylaxis.
Two days after surgery, a severe purulent left knee arthritis developed, in association with local, extensive involvement of soft tissues, and rapidly worsening systemic signs of septic hyperpyrexia and toxaemia. Local and blood cultures yielded a dual staphylococcal infection, confirmed by organism detection from local swabs taken from spontaneous fistulization of the left knee (Staphylococcus aureus), and from concurrent blood cultures (Staphylococcus epidermidis) (Table 1).

In detail, both staphylococci were isolated and identified according to standardized laboratory techniques [26], and proved resistant to several tested compounds, including methicillin (which conferred cross-resistance to all beta-lactams derivatives and other compounds), while they remained sensitive to both glycopeptides, quinupristin/dalfopristin, cotrimoxazole and linezolid in the case of S. aureus, and vancomycin, rifampicin, quinupristin/dalfopristin and linezolid (with borderline “intermediate” teicoplanin and levofloxacin sensitivity), in the case of S. epidermidis.

Table 1 summarizes all minimum inhibitory concentrations (MIC\textsubscript{90}) obtained for each single tested antimicrobial compound, against the two concurrent staphylococcal isolates.

In early phases of infection, pending microbial isolation and in vitro susceptibility assays, em-

<table>
<thead>
<tr>
<th>Tested antimicrobial agents</th>
<th>Staphylococcus aureus (purulent drainage)</th>
<th>Staphylococcus epidermidis (blood cultures)</th>
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<tbody>
<tr>
<td></td>
<td>MIC value (µg/mL)</td>
<td>Interpretation</td>
</tr>
<tr>
<td>Oxacillin (+2% NaCl)</td>
<td>&gt;2</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>&gt;16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Amoxycillin-clavulanate</td>
<td>&gt;4</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Choramphenicol</td>
<td>&gt;16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.25</td>
<td>Resistant</td>
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<tr>
<td>Erythromycin</td>
<td>≤0.25</td>
<td>Resistant</td>
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<tr>
<td>Rifampicin</td>
<td>≥2</td>
<td>Resistant</td>
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<tr>
<td>Gentamicin</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>≤1</td>
<td>Susceptible</td>
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<tr>
<td>Levofloxacil</td>
<td>&gt;4</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>≤0.25</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤1</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>≤0.5</td>
<td>Susceptible</td>
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</table>
pirical regimens based on full-dose i.v. co-amoxiclav and amikacin were started, and surgical debridement disclosed abundant purulent material. Initial antibiotics were replaced three days later with imipenem (2 g/day) associated with repeated local medications, and another surgical curettage of yellowish purulent material, after increased local pain, swelling, local impotence, and overwhelming signs of a systemic septic evolution, as assessed on the ground of very elevated leukocytosis and neutrophilia, and significantly increased erythrocyte sedimentation rate (ESR) and serum C-reactive protein and fibrinogen levels. Finally, on the ground of bacterial isolation and in vitro sensitivity testing, three days later (8 days after surgery), our patient was posed on associated i.v. teicoplanin (800 mg/day) and rifampicin (600 mg/day) therapy and followed at our Division of Infectious Diseases. Unfortunately, despite full-dose combined antimicrobial therapy and daily medications, in absence of further microbiological isolations, no local and systemic improvement was seen, and a further surgical debridement was performed. Radiological imaging (plain X-ray examination, and magnetic resonance imaging -MRI- of the affected knee), showed an extensive involvement of soft tissues, but no sign of osteomyelitis was found.

Ten days after, teicoplanin therapy was replaced with a 24-hour continuous vancomycin infusion at 2 g/day associated with continued full-dose rifampicin, but also this last regimen was interrupted four days later, after evident failure in changing local and systemic signs and symptoms towards an appreciable amelioration: a complete local impotence, severe inflammatory symptoms, abundant purulent discharge, and extending tumefaction of subcutaneous tissues, was accompanied by a steadily elevated body temperature (often exceeding 40°C), and systemic toxaemia.

Finally, full-dose i.v. linezolid treatment was introduced (600 mg twice daily), which prompted a rapid amelioration of local and systemic phlogosis, and a slower defervescence. Five days after linezolid initiation fever disappeared, together with leukocytosis and neutrophilia, while all other inflammatory indexes tended to return to normal values. Immediately after interruption of i.v. linezolid administration (at its 14th day of administration), the day after a novel febrile movement (37.9°C) was observed, so that oral linezolid (at 600 mg twice daily) was re-introduced and continued for further seven days. After the achievement and maintenance of stable defervescence and complete resolution of systemic and local signs and symptoms of infection (which took one week more of daily medications), our patient initiated a rehabilitation program. Two weeks later, our patient could walk with no pain, and reached an almost complete extension of knee joint; after further four weeks, no recrudescence of infection was seen, and after eight weeks our patient is completing the recommended physical medicine program.

**DISCUSSION**

After phase II-III clinical trials and expanded access experiences [7, 16, 17, 27, 28], which showed a favourable activity of open-label linezolid use in the treatment of a broad spectrum of infections caused by gram-positive organisms with variable resistance to methicillin and/or vancomycin, or patients with intolerance or lack of response to other potentially effective treatments, further controlled or at least observational studies were conducted with this novel oxazolidinone compounds [8-11, 19, 29]. A relevant number of clinically and bacteriologically favourable experiences were conducted with linezolid, although the great majority of them lack of randomization or a control group, were conducted on observational basis, often relied on other antimicrobials associated with linezolid, and especially did not specifically focus on bacterial strains who were vancomycin-resistant, but included also methicillin-sensitive, and sometimes penicillin-sensitive isolates, without the possibility to make a separate analysis of the outcome of these different infections, whose common issue was often only failure of previous regimens, and/or linezolid administration.

In particular, a compassionate trial of *S. aureus*-infected patients who were intolerant of, or have failed response to vancomycin, involved 828 episodes of infection (occurred in 797 patients), and bone and joint infections accounted for 27.1% of episodes, thus representing the most frequent cluster, followed by skin and soft tissue infection, bloodstream, and lower respiratory tract infections [28]. In the field of nosocomial pneumonia, efficacy levels of linezolid comparable with those of vancomycin were
demonstrated in a population of 623 patients [8]. Another possible, relevant clinical indication included resistant gram-positive endocarditis [13]. Complicated skin and soft tissue infections [9], either in a direct comparison with oxacillin, or in patients treated with either oxacillin, vancomycin, or linezolid in a design based on either methicillin resistance or susceptibility [10], or in a comparison between oxacillin and linezolid [9, 10], were the target of additional investigation. A further study focused on foot infections in diabetic patients, and demonstrated that linezolid was at least as effective as beta-lactam derivatives protected by beta-lactamase inhibitors, on the basis of a bacterial flora including methicillin-susceptible and -resistant staphylococci, enterococci, and streptococci, with sparse vancomycin-resistant strains [11]. In critically-ill patients, linezolid was compared with teicoplanin in a randomized, double-blind, multicentre experience [29]. Although the study design included other antibiotics and was linezolid- or teicoplanin-dummy, both clinical and bacteriological success rates were comparable (78.9% and 70% for linezolid, and 72.8% and 66.2% for teicoplanin-based therapies, respectively) [29]. Paediatric experiences conducted in comparison with vancomycin confirmed the activity of linezolid against several infections (nosocomial pneumonia, complicated skin-skin structure infections, bacteremia, and other infections sustained by resistant staphylococci, streptococci, and enterococci [12]. Among 321 enrolled patients, the intention-to-treat cure rates and microbiological clearance were slightly greater in the linezolid group, in absence of differences according to patient’s age and infection site. Both tolerability and length of hospitalization were significantly reduced in the linezolid group [12]. Among 63 neonates with known of suspected infection due to gram-positive-resistant organisms, a tendency towards a better clinical and bacteriological response rate was observed in patients with hospital-acquired pneumonia, complicated skin-soft tissue infection, bacteremia, pyelonephritis, or abdominal abscess [22]. When considering bone and joint infections, it becomes extremely important to underline the particularly effective penetration of linezolid into osteo-articular tissues [30]; mean drug concentrations measured 90 minutes after standard dosing were at least twice the serum MIC values for gram-positive organisms in synovial fluid, synovium, muscle and cancellous bone, thus supporting linezolid administration in the management of difficult-to-treat infections of these districts, where antibiotic penetration is often unpredictable, and long-term therapies are frequently needed to clear infection [30]. Further confirmation came from linezolid dosage performed in bone and joint tissues infected with methicillin-resistant staphylococci: mean drug concentrations in the mentioned infected tissues were greater than 10 mg/L, after administration of the preoperative dose [31]. From a clinical point of view, although no large randomized trials have been carried out in patients with orthopaedic infections, data coming from anecdotal cases, small series, observational reports, and open-label comparisons are extremely encouraging [15-20], and stimulate further, controlled clinical and microbiological comparisons, since staphylococci are responsible of nearly 90% of infections of these districts, and their rate of methicillin- and glycopeptide-resistance are increasing.

A first anecdotal case report of successful linezolid treatment of vertebral osteomyelitis due to a persistant methicillin-resistant S. aureus and vancomycin-resistant Enterococcus bacteremia and prior vancomycin-amoxycillin-fusidic acid failure, was reported in a patient undergoing hemodyalisis, five years ago; a comprehensive 4.5-month linezolid treatment plus orthopaedic surgery led to clinical and microbiological success [15].

In a subsequent study, 10.7% of 828 compassionate linezolid courses were favourably delivered for an osteomyelitis mostly caused by methicillin-resistant staphylococci (39 episodes), or vancomycin-resistant Enterococcus faecalis (22 courses) [16]. Moreover, among 25 patients with frequent underlying illnesses and S. aureus infection with reduced vancomycin sensitivity (MIC values ranging from 2 to 4 µg/mL), and previous failure of glycopeptide treatment observed in 79% of cases, six subjects suffered from osteomyelitis or septic arthritis [17], and the switch to linezolid added significantly in all cases, although disease localization, prior treatment, and duration of linezolid therapy were not comparable [17].

An open-label study was recently conducted with oral linezolid in 11 patients (9 with osteomyelitis, and two with septic arthritis), for a mean time of 10 weeks (range 6-19 weeks), with clinical and microbiological success obtained in all cases, in absence of relevant toxicity [18]. In-
volved pathogens included methicillin-resistant staphylococci in 9 cases, and a vancomycin-sensitive and a vancomycin-resistant Enterococcus faecalis in the remaining two patients [18]. A 2000-2002 retrospective survey was aimed to assess linezolid plus surgical therapy in 20 patients with microbiologically-confirmed orthopaedic infections [19]. At a mean follow-up of 276 days, 11 subjects were cured, 7 had clinical improvement but continued on long-term suppressive therapy (always based on combined antimicrobials, including linezolid), one had a relapse after linezolid discontinuation, and the remaining one died due to unrelated causes. Linezolid-related myelosuppression or pancytopenia occurred in overall 50% of cases, and it proved dose-dependent and especially related to treatment duration, so that this last problem was also stressed when prolonged treatments are of possible concern [19, 20].

The authors concluded that linezolid may be an effective alternative antimicrobial treatment for selected patients who are infected with resistant bacterial strains, or cannot tolerate alternative agents [19]. On the ground of clinical experiences carried out in orthopaedics, treatment duration remains a discussed but unresolved problem, since no controlled studies are available, and treated patients are usually not comparable per type and localization of disease, involved organisms, prior treatment, role of surgery or prosthetic implants, re-intervention, and antimicrobial agents eventually associated with linezolid [3, 18-20]. Treatment courses of two to over 6-12 weeks were performed in non-comparable clinical settings, and also the switch from parenteral to oral route was strictly individualized, as well as the duration of maintenance or oral linezolid course.

The presented case report of a septic post-surgical staphylococcal arthritis is particularly intriguing, due to its lack of response to associated glycopeptide administration, despite an apparently favourable in vitro susceptibility assay (when excluding a nearly “intermediate” teicoplanin sensitivity of a haematogenous S. epidermidis), and the unexpectedly rapid response to i.v. followed by oral administration of linezolid (21 overall days of therapy). The absence of bone involvement or abscess formation (as demonstrated by radiological and MRI studies), and the extensive soft tissue infection were not expected to represent an obstacle to full-dose glycopeptide-rifampicin activity, while also in vitro MIC$_{90}$ values showed susceptibility of S. aureus to vancomycin and teicoplanin, and sensitivity of an accompanying Staphylococcus epidermidis isolated from blood cultures to vancomycin and rifampicin, with borderline “intermediate” values found for teicoplanin only.

In particular, the methicillin- and rifampicin-resistant strain of S. aureus recovered from purulent joint material showed MIC$_{90}$ values $\leq$0.25 $\mu$g/mL for quinupristin-dalfopristin, $\leq$0.5 $\mu$g/mL for teicoplanin, $\leq$1.0 $\mu$g/mL for linezolid and cotrimoxazole, and =1.0 $\mu$g/mL for vancomycin, while the methicillin- and cotrimoxazole-resistant S. epidermidis strain from blood cultures was susceptible to rifampicin (with a MIC$_{90}$ value $\leq$1.0 $\mu$g/mL), while it tested borderline “intermediate” to both levofloxacin (MIC$_{90}$ 4 $\mu$g/mL), and also teicoplanin (MIC$_{90}$ 4 $\mu$g/mL) (Table 1).

From a strictly clinical point of view, the introduction of a two-week i.v. linezolid followed by one more week of oral linezolid led to complete clinical and microbiological cure, and a very favourable and unexpectedly rapid functional outcome, which has no evident explanation in the light of prior, severe clinical resistance and worsening infection, under glycopeptide-rifampicin association.

Also based on our direct experience of the unusual presented case report, i.v. and oral linezolid (as well as quinupristin-dalfopristin) [5, 6], represent promising antimicrobial agent and provide the clinician with additional, sometimes life- and function-saving treatment option, especially among the limited therapies for resistant gram-positive bacterial infections in critical care or critically-ill patients. When waiting for other molecules currently on development (including daptomycin, tigecycline, and oritavancin), updated recommendations for a rational use of linezolid are strongly needed, in order to preserve the efficacy of this novel, key antimicrobial agent for severe multiresistant gram-positive infections.

In the field of bone and joint infection, controlled clinical trials are strongly needed in order to support writing updated guidelines of antimicrobial therapy, and to ensure an adequate exploitation of these innovative antimicrobial resources.

Key words: Joint infection, staphylococci, glycopeptides, rifampicin, linezolid
SUMMARY

We present an intriguing case report of a septicemic post-elective surgical Staphylococcal knee arthritis and cellulitis which did not respond to long-term courses of associated rifampicin and teicoplanin or vancomycin despite apparently favourable *in vitro* susceptibility assays, but rapidly resolved after i.v. followed by oral administration of linezolid. The lack of response to a two-week course of glycopeptides cannot be explained by the *in vitro* minimum inhibitory concentrations (MICs) of involved organisms, which showed full susceptibility of *Staphylococcus aureus* to vancomycin and teicoplanin, and sensitivity of an accompanying *Staphylococcus epidermidis* isolated from blood cultures to vancomycin and rifampicin, with “intermediate” values found for teicoplanin. Since neither abscess formation nor bone involvement were of concern, effective glycopeptide and rifampicin penetration into infectious tissue should have been ensured. From a clinical point of view, only the introduction of a two-week i.v. linezolid followed by one more week of oral linezolid obtained a complete clinical and microbiological cure, and an unhoped-for functional success. When managing severe multiresistant gram-positive infections, *in vitro* activity should be carefully evaluated against expected drug penetration rates into the relevant infectious tissues.

REFERENCES