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Efficacy and safety of meropenem/clavunate added to linezolid containing regimens in the treatment of M/XDR-TB

De Lorenzo Saverio*[1], Alffenaar Jan Wilem*[2], Sotgiu Giovanni[3], Centis Rosella[4], D'Ambrosio Lia[4], Tiberi Simon[1], Bolhuis Mathieu S[2], van Altena Richard[6], Viggiani Piero[1], Piana Andrea[3], Spanevello Antonio[5], Migliori Giovanni Battista[4]

**Equally contributed

1 AOVV E. Morelli Hospital, Reference Hospital for MDR and HIV-TB, Sondalo, Italy;

2 University of Groningen, University Medical Center Groningen, Department of Hospital and Clinical Pharmacy, Groningen, the Netherlands;

3 Epidemiology and Medical Statistics Unit, Department of Biomedical Sciences, University of Sassari, Sassari, Italy;

4 World Health Organization Collaborating Centre for Tuberculosis and Lung Diseases, Fondazione S. Maugeri, Care and Research Institute, Tradate, Italy;

5 Università degli Studi dell'Insubria, Varese, and Fondazione S. Maugeri, Care and Research Institute, Tradate, Italy;

6 University of Groningen, University Medical Center Groningen, Tuberculosis Center Beatrixoord, Haren, the Netherlands;

Correspondence: G.B. Migliori, World Health Organization Collaborating Centre for Tuberculosis and Lung Diseases, Fondazione S. Maugeri, Care and Research Institute, Via Roncaccio 16, 21049, Tradate, Italy. E-mail: giovannibattista.migliori@fsm.it

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Summary (200 words)

Clinical experience on meropenem/clavulanate (MC) to treat tuberculosis (TB) is anedoctal (case-reports on 10 patients). Aim of our case-control study was to evaluate the contribution of MC when added to linezolid-containing regimens in terms of efficacy, safety/tolerability in treating multidrug-(MDR-) and extensively drug-resistant (XDR-) TB cases after 3 months of second-line treatment.

Cases with MDR/XDR-TB (37) were prescribed MC (daily dose: 3 g) in addition to a linezolid-containing regimen (dosage range: 300-1,200 mg/day), designed according to international guidelines, which was prescribed to controls (61).

The clinical severity of cases was worse than that of controls (drug susceptibility profile; proportion of sputum smear positive and of re-treatment cases). The group of cases yielded a higher proportion of sputum smear converters (28/32, 87.5% VS. 9/16, 56.3%; p-value: 0.02) and culture converters (31/37, 83.8% VS. 15/24, 62.5%; p-value: 0.06). Excluding XDR-TB patients (11/98, 11.2%), cases scored a significantly higher proportion of culture converters than controls (p-value: 0.03).
One case had to withdraw MC due to increased transaminase levels.

The results of our study provide: 1) preliminary evidence on effectiveness and safety/tolerability of MC; 2) reference to design further trials and 3) guide to clinicians for its rationale use within salvage /compassionate regimens.

Introduction

According to the World Health Organization (WHO) an estimated 650,000 prevalent cases of multidrug-resistant tuberculosis (MDR-TB) occurred globally in 2010 [1-4].
The dimension of the problem is alarming, as 12 countries have reported nationwide or sub-national proportions of MDR-TB of 6% or more among new tuberculosis (TB) cases and 5 of these countries also reported MDR-TB proportions of 50% or more among previously treated cases [1-5]. In Minsk (Belarus) over 50% of TB cases are presently affected by MDR-TB [6].
WHO estimated 290,000 new cases of MDR-TB among notified cases of pulmonary TB in 2010, of whom only around 50,000 were reported to have been enrolled on treatment [1-3,5].
Overall, 5.4% of MDR-TB cases were found to be affected by extensively drug-resistant (XDR-) TB strains of *M. tuberculosis* [1-3,5]. Furthermore, new strains which are resistant to all drugs tested have been described in Italy, Iran and India [7-9], and the international community is still discussing whether the proposed acronym of TDR (totally drug resistant)-TB is adequate to describe the patterns of drug resistance beyond XDR-TB [10].
The difficulty to identify at least 4 active drugs, suitable to be included in a multi-drug regimen effective against these complicated forms of TB according to WHO recommendations, makes the need for new antibiotics really urgent [11] .
Three new drugs, presently in the development pipeline (delamanid, bedaquiline and PA-824), have given preliminary evidence of their efficacy [1,12,13].

Delamanid in combination with a background regimen, developed according to the WHO guidelines, is associated with an increase in sputum-culture conversion at 2 months in patients with MDR-TB [11,12].

A recent multiple-agent combination study assessed that the 14-day early bactericidal activity of Pa-824+moxifloxacin+pyrazinamide resulted significantly higher than that of bedaquiline, bedaquiline+pyrazinamide, Pa-824+pyrazinamide and bedaquiline+Pa-824 and comparable to that of the standard treatment regimen of isoniazid, rifampicin and pyrazinamide with streptomycin or ethambutol [13].

In parallel, with further research on the best way to combine these new drugs into new regimens, additional evidence is necessary to confirm the clinical usefulness of existing drugs, presently used "off label" to manage difficult-to-treat MDR- and XDR-TB cases.

Mounting evidence indicates that the efficacy of linezolid is limited by its toxicity [14, 15].

The association of a β-lactam antibiotic with a β-lactamase inhibitor has been explored recently with apparently sub-optimal results, as *M. tuberculosis* is protected from β-lactams antibiotics through its potent β-lactamase, encoded by a gene located in the chromosome and called BlaC [16,17]. Fortunately, clavulanate (a β-lactamase inhibitor) has demonstrated *in vitro* the capacity to inhibit the activity of BlaC-coded products [18]. Meropenem, a carbapenem offering a limited substrate to hydrolysis, has demonstrated high bactericidal *in vitro* activity when combined to clavulanate against susceptible, MDR- and XDR-TB strains of *M. tuberculosis* and the capacity to sterilize cultures *in vivo* within 2 weeks [19,20].

At present the clinical experience on meropenem/clavulanate (MC) is anecdotal, and limited to case-reports of no more than 10 patients where the drug was prescribed for salvage purposes [16,19].

Taking advantage of the existing collaboration and established research protocols, based on WHO guidelines, in TB reference centres in Sondalo, Italy, and in Haren, the Netherlands [11,15], a study was conducted to evaluate the therapeutic contribution of MC when added to linezolid-containing regimens during hospital stay in terms of efficacy as well as safety and tolerability profile in treating MDR- and XDR-TB cases after 3 months of therapy with second-line drugs.

Material and Methods

An observational case-control study was carried out in two centres specialized in the management of complicated TB cases.

Adult patients only (*i.e.*, aged ≥15 years) with culture-confirmed MDR-TB (*i.e.*, TB caused by *M. tuberculosis* isolates resistant to at least isoniazid and rifampicin) were consecutively selected and enrolled in both centres.

Resistance to first- and second-line anti-TB drugs was assessed by the quality-assured laboratories located in both centres and confirmed by the WHO Supranational Reference Laboratories of Milan, in Italy, and the National Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, in The Netherlands.

On the basis of the drug-susceptibility test (DST), anti-TB regimens were administered following the WHO recommendations [11].

Both centres pioneered the off-label use of linezolid between 2001 and 2004. Clinicians working in Sondalo started to prescribe MC in 2009.

Cases were individuals treated with an anti-TB MC-containing regimen, which also included linezolid in all but 5 patients. The cases were included in the Italian cohort, treated in Sondalo Hospital.

The reasons for not prescribing linezolid were the following: availability of 4 effective drugs (3 cases), lack of DST on linezolid in the presence of 4 effective alternative drugs (1 case) and concurrent anaemia (1 case).

Controls were subjects treated in Haren hospital, whose linezolid-containing regimen did not include MC. MC was prescribed intravenously at a dosage of 1 g three times a day whereas linezolid was given at a dosage ranging from 300 to 1,200 mg per day after adjusting the dose based on blood levels. The ratio between cases and controls was 1:2. Drugs' prescription was not blinded or randomized, following only the DST results as per WHO guidelines [11].

Ethical approval for the collection and analysis of anonymous and retrospective data and for the compassionate use of the drugs was obtained by institutional review boards of the participating institutions as per legislation in force (formal approval not needed in the Netherlands).

Epidemiological, clinical, microbiological information was collected from clinical files on standardized *ad-hoc* e-forms. In particular, the following covariates were recorded: date of admission, date and place of birth, gender, residence, immigration from a TB high-burden country, HIV-positivity, exposure to antiretroviral drugs, previous TB diagnoses, previous anti-TB treatments (*i.e.,* exposure to anti-TB drugs for more than one month) and previous treatment outcomes, DST results, including susceptibility or resistance to the drugs defining XDR-TB (*i.e.*, TB caused by *M. tuberculosis* strains resistant to isoniazid, rifampicin, any fluoroquinolones, and at least one second-line injectable anti-TB drug –amikacin, capreomycin, kanamycin-), radiological findings, anti-TB regimen administered (including dosage and length of exposure), surgery, duration of exposure to linezolid and MC, adverse events potentially ascribed to the study drugs (*i.e.*, linezolid and MC), management of adverse events, proportion of sputum smear and culture positivity at the hospital admission and at 30, 60 and 90 days after the prescription of second-line anti-TB drugs, time to sputum smear and culture conversion.

Qualitative and not normally distributed quantitative variables were statistically evaluated with the χ^2 and the Wilcoxon-Mann-Whitney tests, respectively; normality distribution of the continuous data was tested with the Shapiro-Wilk test.

P-values >0.05 were considered not statistically significant.

All the statistical computations were performed using the statistical software Stata 9.0 (StataCorp, College Station, TX).

Results

Ninety-eight MDR-TB patients were enrolled in the study: 37 (cases) and 61 (controls) were treated, respectively, with individualized anti-TB MC- containing and -sparing regimens (Table 1). The former were managed in the Italian reference centre in Sondalo, whereas the latter in the Dutch reference centre in Haren.

Almost 60% were male, with a median (InterQuartile Range-IQR-) age of 30 (24-38) years.

Most of them were migrants (96/98, 98.0%), coming from Europe (48/98, 49%), Africa (26/98, 26.5%) and Asia (19/98, 19.4%). The percentage of TB/HIV co-infected patients was <10%, and 75% of them were treated with antiretroviral drugs.

No statistical differences between group and control cases regarding social and demographic variables were detected, except for the European origin, being significantly higher among cases treated with a MC-containing regimen (29/37, 78.4% VS. 21/61, 34.4%; p-value: <0.0001).

The majority showed pulmonary TB disease (89/98, 90.8%), with only 12% affected by an extra-pulmonary TB form. One third presented bilateral pulmonary involvement with cavitary lesions, while one third showed unilateral infiltrates. Half of the patients were sputum smear positive at hospital admission, with a statistically significant higher prevalence among cases (32/37, 86.5% VS. 18/61, 29.5%; p-value: <0.0001). Overall, almost 13% of the cases enrolled underwent surgical intervention. The proportion of patients who were prescribed previous anti-TB treatment was higher among the cases treated with MC (58.3%, 21/36) than among the controls (32.8%, 20/61).

Cases treated with a MC-containing regimen were infected by *M. tuberculosis* strains showing a worse drug-susceptibility profile: the proportion of XDR-TB cases was significantly higher (9/37, 24.3% VS. 2/61, 3.3%; p-value: 0.001) as well as the percentage of those harbouring *M. tuberculosis* strains resistant to pyrazinamide (31/36, 86.1% VS. 18/54, 33.3%; p-value: <0.0001), fluoroquinolones (15/37, 40.5% VS. 6/60, 10%; p-value: <0.0001), ethionamide (24/36, 66.7% VS. 9/69, 15%; p-value: <0.0001), and cycloserine (8/34, 23.5% VS. 2/39, 5.1%; p-value: 0.02).

In addition, cases were more likely to be previously treated (21/36 -58.3% VS. 20/61 - 32.8%; p-value: 0.01) and to be born in Eastern European countries (28/37 - 75.7% VS. 17/61 -27.9%; p-value: <0.0001).

Efficacy analysis.

Patients were treated with a linezolid-containing regimen for a median (IQR) time of 61 (35-117) days. Individuals admitted to the Italian hospital (cases) were exposed to a MC-containing regimen for a median (IQR) period of 67.0 (46.0-85.0) days. Duration of linezolid and/or MC exposure was determined by several parameters, including clinical improvement, sputum smear and/or culture conversion and occurrence of life-threatening adverse events.

No significant statistical differences were found in the median time to sputum smear and culture conversion between cases and controls (46 VS. 52.5 days and 46 VS. 42 days, respectively) (Table 2).

After 90 days of treatment with second-line drugs the proportion of sputum smear converters was significantly higher in the MC-treated patients' group (28/32, 87.5% VS. 9/16, 56.3%; p-value: 0.02) while the percentage of culture converters reached a borderline statistical significance (31/37, 83.8% VS. 15/24, 62.5%; p-value: 0.06).

Similar results were obtained after the exclusion of the five MC-treated patients not exposed to linezolid (Table 4).

A sub-analysis, which excluded the XDR-TB patients (11/98, 11.2%) to correct partly the worse DST pattern of cases when compared to controls, showed that a significant higher percentage of cases exposed to MC achieved sputum culture conversion (p-value: 0.03; Figure 1).

Safety and tolerability analysis.

Fifteen out of thirty-seven (40.5%) cases and seven out of sixty-one (11.5%) controls experienced adverse reactions following drug prescription (p-value: 0.001). The higher proportion of adverse events among cases was almost entirely related to linezolid (12/32, 37.5% VS. 7/61, 11.5%; p-value: 0.003), reckoned to be the consequence of exposure to a superior dosage (>600 mg/ day) of linezolid (21/32, 65.6% VS. 18/61, 29.5%; p-value: 0.001).

Thirteen percent (12/93) of the selected patients managed in both centres experienced adverse events potentially caused by linezolid which required interruption of the drug (Table 3).

Those treated with a daily linezolid dosage of ≤600 mg interrupted their regimen less frequently due to adverse effects attributable to linezolid when compared with patients exposed to a daily dosage

of >600 mg (3/54, 5.6% VS. 9/39, 23.1%; p-value: 0.01). No statistical differences between patients treated with <600 mg and >600 mg were found with regard to anaemia, leukopenia, peripheral neuropathy and gastro-intestinal disorders.

Only 5 patients out of 37 (13.5%) experienced adverse events potentially attributed to MC. In all cases diarrhoea was present, and did not require withdrawal of the drug. In addition, two out of the 5 cases experienced transient increase of liver function tests. MC was stopped and after one week re-started. While in one case MC was continued without problems, the other case saw a renewed increase in transaminase levels, the drug was stopped, and transaminases normalised rapidly.

Discussion

The aim of the present research was to assess the therapeutic contribution of MC when added to linezolid-containing regimens during hospital stay in terms of efficacy, safety and tolerability in treating MDR- and XDR-TB cases after 3 months of treatment with second-line drugs.

To our knowledge, this is the first large study evaluating the added value of MC in managing difficult-to-treat M/XDR-TB cases.

In spite of the worse initial clinical severity of cases (in terms of proportion of re-treatment cases, XDR-TB cases, proportion of sputum smear positive cases and prevalence of resistance to pyrazinamide, fluoroquinolones, ethionamide, and cycloserine), MC increased significantly the proportion of sputum smear conversion in the overall sample, including XDR-TB patients, and of culture conversion among MDR-TB cases after 90 days of treatment with second-line drugs.

Furthermore, although the clinical pattern of cases was significantly worse than that of controls (as previously mentioned) the added value of MC has been seen already after 60 days of treatment with second-line drugs (with differences not yet significant) although no difference was found in the time to microbiological conversion.

While a comprehensive assessment of culture conversion was done, we could not unfortunately assess the sputum smear conversion in all the individuals. This was probably due to a lower pulmonary bacillary load in those with a less severe disease (particularly in the control group) and to the effect of previous treatments patients underwent before being admitted at the reference centres.

Importantly, MC at an intra-venous dosage of 3 g once a day was well tolerated and a single episode of drug withdrawal was recorded over a median hospital exposure time of 67 days.

The clinician's decision to interrupt MC (a drug administered intravenously only and needing in-patient care) was related to clinical improvement and microbiological conversion (e.g. the conditions allowing hospital discharge) and not to adverse events occurrence in all but one case.

Our study has provided, for the first time, evidence that MC increases the proportion of microbiological converters when added to linezolid-containing regimens, designed according to WHO guidelines [11]. The large sample selected allowed to obtain more statistical confidence than in previously published case series. In addition, the study was conducted in two specialised centres having collaborated in the past [15,21] and sharing the same protocol to design linezolid-containing regimens and to routinely adjusting the linezolid dose based on blood levels [15].

The study protocol allowed collection of quality-assured patients' information in both centres, making a comprehensive comparison between case and control groups possible.

A further finding of the study is the possibility to add evidence on safety and tolerability of linezolid, taking advantage of 93 cases not included in a recently published individual meta-analysis [15]. While the proportion of major adverse events was lower than previously reported (12.9%), the study results confirm the better safety/tolerability of the lower dose (≤600 mg a day) of linezolid [14,15], prescribed more frequently in the Dutch centre and responsible for the significantly lower proportion of total adverse events observed among controls.

Among the main methodological limitations it is worthwhile to mention the observational, retrospective nature of the study and the consecutive, not randomized enrolment of patients.

In addition, although quality-controlled by Supranational Reference Laboratories, and distributed among both cases and controls, DSTs for some of the second-line drugs (e.g. cycloserine) need to be evaluated with caution, given the intrinsic difficulty to perform these tests.

Although the sample size allowed inferential analysis, larger trials will shed further light on the definite role that MC might play in treating M/XDR-TB cases (e.g., duration of exposure according to clinical severity) when a fourth active drug is needed to design an effective regimen [11].

Although evidence on three new drugs (delamanid, bedaquiline and PA-824) is rapidly accumulating, preceding their market introduction [22], the WHO Group V drugs are still of interest in the management of complicated cases when a fourth active drug is not available either

because of the DST profile or because of intolerance to active drugs [11]. On top of continuing research on newly developed drugs and regimens, parallel evidence needs to be raised on existing compounds on which little is proven.

Considering the promising safety/tolerability and efficacy profile of MC found in our study, further research on its role and contribution in managing M/XDR-TB cases should be encouraged. Furthermore, the 2012 daily cost of MC (in Sondalo, 1 g vial three times a day for intravenous use: ~57,00 €) is substantially similar to that of linezolid (600 mg tablet: ~55,00 € in Sondalo and ~61,00 € in Haren) making its use potentially affordable at least in high income countries.

The results of the present study will provide reference to design trials on MC and will guide clinicians for its rationale use within salvage/compassionate regimens [25-26].

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Table 1. Epidemiological and clinical characteristics of 98 MDR-TB cases enrolled in two specialized clinical centres in Italy and in the Netherlands.

Variables		Total	Cases Meropenem-containing anti-TB regimen -Italian cohort	Controls Meropenem/clavulanate-sparing anti-TB regimen -Dutch cohort	p-value
Male, n (%)		58/98 (59.2)	25/37 (67.6)	33/61 (54.1)	0.19
Median (IQR) age at the admission, years		30 (24-38)	32 (26-41)	29 (24-35)	0.21
Country of birth, n (%)	*Europe*	48/98 (49)	29/37 (78.4)	21/61 (34.4)	<0.0001
	Asia	19/98 (19.4)	2/37 (5.4)	15/61 (24.6)	0.02
	Africa	26/98 (26.5)	5/37 (13.5)	21/61 (34.4)	0.02
	Other geographical areas	5/98 (5.1)	1/37 (2.7)	4/61 (6.6)	0.40
Migrant, n (%)		96/98 (98.0)	36/37 (97.3)	60/61 (98.4)	0.72
HIV-positive, n (%)		8/88 (9.1)	4/37 (10.8)	4/51 (7.8)	0.63
Exposure to ART, n (%)		6/8 (75)	3/4 (75)	3/4 (75)	-
Previous exposure to anti-TB therapy > 1 month, n (%)		41/97 (42.3)	21/36 (58.3)	20/61 (32.8)	0.01
Median (IQR) number of times treated with anti-TB drugs for > 1 month		1 (1-2)	1 (0-2)	1 (1-1)	0.99
Sputum smear positives, n (%)		50/98 (51.0)	32/37 (86.5)	18/61 (29.5)	<0.0001
Pulmonary TB, n (%)		89/98 (90.8)	34/37 (91.9)	55/61 (90.2)	0.77
Extra-pulmonary TB, n (%)		12/98 (12.2)	5/37 (13.5)	7/61 (11.5)	0.77
Radiological findings, n (%)	*Cavitary lesions*	17/89 (19.1)	8/34 (23.5)	9/55 (16.4)	0.41
	Bilateral pulmonary involvement with cavitary lesions	29/89 (32.6)	11/34 (32.4)	18/55 (32.7)	0.98
	Bilateral pulmonary involvement	13/89 (14.6)	6/34 (17.7)	7/55 (12.7)	0.52
	Non-cavitary unilateral pulmonary involvement	30/89 (33.7)	9/34 (26.5)	21/55 (38.2)	0.26
XDR-TB, n (%)		11/98 (11.2)	9/37 (24.3)	2/61 (3.3)	0.001
Resistance to ethambutol, n (%)		66/97 (68.0)	29/37 (78.4)	37/60 (61.7)	0.09
Resistance to pyrazinamide, n (%)		49/90 (54.4)	31/36 (86.1)	18/54 (33.3)	<0.0001
Resistance to fluoroquinolones, n (%)		21/97 (21.7)	15/37 (40.5)	6/60 (10.0)	<0.0001
Resistance to ethionamide, n (%)		33/96 (34.4)	24/36 (66.7)	9/60 (15.0)	<0.0001
Resistance to cycloserine, n (%)		10/73 (13.7)	8/34 (23.5)	2/39 (5.1)	0.02

Resistance to amikacin, n (%)	20/97 (20.6)	10/37 (27.0)	10/60 (16.7)	0.22
Resistance to capreomycin, n (%)	18/61 (29.5)	12/37 (32.4)	6/24 (25.0)	0.53
Resistance to kanamycin, n (%)	23/62 (37.1)	16/37 (43.2)	7/25 (28.0)	0.22
Surgical treatment, n (%)	12/96 (12.5)	7/35 (20.0)	5/61 (8.2)	0.09
Median (IQR) hospital stay, days	89.5 (61-153)	81 (60-112)	91 (75-197)	0.03
Median (IQR) exposure to linezolid, days	61 (35-117)	79.0 (50.5-133.0)	59.0 (34.0-93.0)	0.08
Median (IQR) exposure to meropenem, days	-	67.0 (46.0-85.0)	-	-

TB: tuberculosis

IQR: Inter-Quartile Range

HIV: Human Immunodeficiency Virus

ART: Anti-Retroviral Therapy

XDR-TB: Extensively Drug-Resistant tuberculosis

Table 2. Treatment outcomes of 98 MDR-TB cases enrolled in two specialized clinical centres in Italy and in the Netherlands after 30, 60 and 90 days of treatment.

Variables	Total	Cases Meropenem/clavulanate - containing anti-TB regimen -Italian cohort	Controls Meropenem/clavulanate - sparing anti-TB regimen -Dutch cohort	p-value
Sputum smear conversion at 30 days of treatment, n (%)	16/50 (32.0)	7/32 (21.9)	9/18 (50.0)	0.04
Sputum smear conversion at 60 days of treatment, n (%)	27/48 (56.3)	20/32 (62.5)	7/16 (43.8)	0.22
Sputum smear conversion at 90 days of treatment, n (%)	37/48 (77.1)	28/32 (87.5)	9/16 (56.3)	0.02
Culture conversion at 30 days of treatment, n (%)	24/66 (36.4)	12/37 (32.4)	12/29 (41.4)	0.45
Culture conversion at 60 days of treatment, n (%)	37/62 (59.7)	24/37 (64.9)	13/25 (52.0)	0.31
Culture conversion at 90 days of treatment, n (%)	46/61 (75.4)	31/37 (83.8)	15/24 (62.5)	0.06
Median (IQR) period from start of anti-TB therapy to sputum smear conversion, days	51 (28.0-75.0)	52.5 (38.5-65.0)	46.0 (6.0-157.0)	0.85
Median (IQR) period from start of anti-TB therapy to culture conversion, days	42 (16.5-82.0)	42.0 (28.0-65.0)	46.0 (13.0-96.0)	0.96

TB: tuberculosis

IQR: Inter-Quartile Range

Table 3. Safety and tolerability of linezolid in 93 MDR-TB cases.

Variables	Total	Cohort treated with Linezolid ≤600 mg/day n= 54	Cohort treated with Linezolid >600 mg/day n= 39	p-value
Median (IQR) exposure to linezolid, days	61 (35-117)	63.5 (46.0-120.0)	52.0 (30.0-100.0)	0.12
Interruption of Linezolid due to adverse events, n (%)	12/93 (12.9)	3/54 (5.6)	9/39 (23.1)	0.01
Median (IQR) time of occurrence of linezolid-related adverse events, days	32.0 (21.5-62.5)	27.5 (15.5-38.0)	53.0 (37.5-83.0)	0.005
Reversible adverse events, n (%)	13/19 (68.4)	2/6 (33.3)	11/13 (84.6)	0.03
Anaemia, n (%)	13/56 (23.2)	5/24 (20.8)	8/32 (25.0)	0.72
Leucopoenia, n (%)	4/68 (5.9)	2/35 (5.7)	2/33 (6.1)	0.95
Thrombocytopenia, n (%)	4/79 (5.1)	1/45 (2.2)	3/34 (8.8)	0.19
Peripheral neuropathy, n (%)	17/54 (31.5)	8/24 (33.3)	9/30 (30.0)	0.79
Optic neuritis, n (%)	1/79 (1.3)	1/44 (2.3)	0/35 (0.0)	0.37
Gastro-intestinal disorders, n (%)	5/38 (13.2)	2/12 (16.7)	3/26 (11.5)	0.66

TB: tuberculosis

IQR: Inter-Quartile Range

Table 4. Treatment outcomes of MDR-TB patients, excluding those not treated with linezolid, enrolled in two specialized clinical centres in Italy and in the Netherlands after 30, 60 and 90 days of treatment.

Variables	Cases Meropenem/clavulanate-containing anti-TB regimen -Italian cohort	Controls Meropenem/clavulanate -sparing anti-TB regimen -Dutch cohort	p-value
Sputum smear conversion at 30 days of treatment, n (%)	5/28 (17.9)	9/18 (50.0)	0.02
Sputum smear conversion at 60 days of treatment, n (%)	17/28 (60.7)	7/16 (43.8)	0.28
Sputum smear conversion at 90 days of treatment, n (%)	24/28 (85.7)	9/16 (56.3)	0.03
Culture conversion at 30 days of treatment, n (%)	9/32 (28.1)	12/29 (41.4)	0.28
Culture conversion at 60 days of treatment, n (%)	20/32 (62.5)	13/25 (52.0)	0.43
Culture conversion at 90 days of treatment, n (%)	26/32 (81.3)	15/24 (62.5)	0.12
Median (IQR) period from start of anti-TB therapy to sputum smear conversion, days	52.5 (42.0-65.0)	46.0 (6.0-157.0)	0.80
Median (IQR) period from start of anti-TB therapy to culture conversion, days	42.0 (28.0-77.0)	46.0 (13.0-96.0)	0.79

TB: tuberculosis

IQR: Inter-Quartile Range

Figure 1. Bacteriological outcomes at 90 days of MDR-TB cases, excluding the XDR-TB patients, enrolled in two specialized clinical centres in Italy (black bars) and in the Netherlands (grey bars) .