In vitro activity of cefoxitin and imipenem against *Mycobacterium abscessus* complex

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Abstract

The *in vitro* activity of cefoxitin and imipenem was compared for 43 strains of the *Mycobacterium abscessus* complex, mostly isolated from cystic fibrosis patients. The MICs of imipenem were lower than those of cefoxitin, although the number of imipenem-resistant strains was higher according to the CLSI breakpoints. Strain comparisons indicated that the MICs of cefoxitin were significantly higher for *Mycobacterium bolletii* than for *M. abscessus*. The MICs of both β-lactams were higher for the rough morphotype than for the smooth morphotype. The clinical impact of the *in vitro* difference between the activity of imipenem and that of cefoxitin remains to be determined.

Keywords: Antimicrobial susceptibility, cefoxitin, cystic fibrosis, imipenem, *Mycobacterium abscessus*
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Mycobacterium abscessus is the rapidly growing mycobacterium most frequently recovered from pulmonary infections, particularly in cystic fibrosis patients [1,2]. *M. abscessus* is known to be one of the most drug-resistant mycobacteria, and the optimal treatment of lung disease, including its duration, has not yet been formally established [3,4].

Clinical isolates of *M. abscessus* have been assigned to three species on the basis of the *rpoB* sequence: *M. abscessus* sensu stricto, *Mycobacterium bolletii*, and *Mycobacterium massiliense* [5,6]. Recently, *M. massiliense* and *M. bolletii* have been united and reclassified as *M. abscessus* ssp. *bolletii* comb. nov. [7]. However, the distinction between *M. bolletii* and *M. massiliense* is clinically relevant, as an *erm* gene confers inducible resistance to macrolides in *M. bolletii* (and *M. abscessus*) but not in *M. massiliense* [8,9]. In clinical studies, rates of response to clarithromycin-based antibiotic combinations were significantly higher in pulmonary infections caused by *M. massiliense* than in those caused by *M. abscessus* [10,11]. Thus, expression of the *erm* gene further limits the therapeutic options for *M. abscessus* and *M. bolletii* infections. On the basis of limited clinical evidence, many cystic fibrosis centres have abandoned clarithromycin, and use two parenteral agents: an intravenous β-lactam, either imipenem or cefoxitin, combined with amikacin [3,12].

The *in vitro* activity of cefoxitin and imipenem has been previously evaluated [11], but the three species of the *M. abscessus* complex have not been extensively compared. In the present study, we determined the MICs and MBCs of cefoxitin and imipenem for 43 unrelated clinical strains of the *M. abscessus* complex, mostly isolated from cystic fibrosis patients, and characterized by multilocus sequence typing. We also evaluated the impact of colony morphotype, growth phase and culture medium on the activity of these β-lactams.

The 43 clinical isolates of *M. abscessus* were previously collected from 12 French hospitals and one German hospital between 1997 and 2008 [13]. Multilocus sequence typing showed that these isolates are unrelated, and include representatives of the *M. abscessus* complex (15 *M. abscessus*, 14 *M. bolletii*, and 14 *M. massiliense*) [13]. Forty-two strains were isolated from respiratory secretions from 41 cystic fibrosis patients, who met the diagnostic criteria for non-tuberculous mycobacterial respiratory disease [3], and from one human immunodeficiency virus patient. The remaining isolate was recovered from synovial fluid. The reference strains *M. abscessus* CIP 104536^T, *M. bolletii* CIP 108541^T, *M. massiliense* CIP 108297^T and *Staphylococcus aureus* CIP 4.83 were used as controls.

Susceptibility testing of cefoxitin (Panpharma, Fougères, France) and imipenem (MSD, Courbevoie, France) was performed according to the CLSI guidelines [14], except that resazurin was used to facilitate reading. Briefly, the broth microdilution method was performed in cation-adjusted Mueller–Hinton Broth (CAMHB) with an inoculum of

ca. 5×10^5 CFU/mL in the exponential phase of growth. After 3 days of incubation at 30°C, 5 μL of a 0.025% (w/v) resazurin solution (Sigma-Aldrich, Saint-Quentin Fallavier, France) was added to each well (100 μL), and incubation was continued for 24 h at 30°C [15]. Middlebrook 7H9 (BD-Difco, Le Pont de Claix, France) broth supplemented with 10% (v/v) oleic acid, dextrose and catalase (BD-Difco) and 0.05% (v/v) Tween-80 (Sigma-Aldrich; 7H9c medium) was tested in the same conditions, except that resazurin was added after 2 days of incubation. MBCs were determined by plating 100 μL of a 1:10 dilution from each well onto Brain Heart Infusion agar plates. Plates were incubated at 30°C for 7 days, and the MBC was defined as the lowest antibiotic concentration that reduced bacterial counts by at least 99.9% in comparison with the inoculum. Reference strains were included in each plate as controls (data presented in Table S1).

In CAMHB, MICs of cefoxitin were significantly higher for *M. bolletii* than for *M. abscessus* (p 0.006; Mann–Whitney *U*-test; Table 1), whereas no difference was observed between *M. bolletii* and *M. massiliense* (p 0.13) or between *M. abscessus* and *M. massiliense* (p 0.46). Imipenem was similarly active against the three species (p 0.20, Kruskall–Wallis test). MICs of imipenem were lower than those of cefoxitin for the three species ($p < 10^{-4}$, Mann–Whitney *U*-test), but more isolates were resistant to imipenem than to cefoxitin according to CLSI breakpoints [14] (Table 1). Susceptibilities to imipenem and cefoxitin were correlated with a Pearson correlation coefficient of 0.74 (Fig. S1), implying that strains with a high cefoxitin MIC were likely to have a high imipenem MIC. The addition of 4 mg/L clavulanate (kindly provided by GSK, Marly-le-Roi, France) did not change the MIC values.

According to CLSI breakpoints [14], all *M. abscessus* and *M. massiliense* isolates were susceptible or intermediately resistant to cefoxitin (Table 1). One *M. bolletii* isolate was resistant to this drug. Two *M. abscessus*, one *M. bolletii* and four *M. massiliense* isolates were resistant to imipenem. Imipenem resistance rates of 19% and 48% have been previously reported for *M. abscessus* and *M. massiliense*, respectively [11].

Clinical isolates of *M. abscessus* show a smooth or rough colony morphotype, owing to the loss of glycopeptidolipid production by the latter variants [16]. In humans, smooth variants of *M. abscessus* are found at early stages of the infection, whereas rough variants generally appear several years later, and the morphological switch has been linked to clinical deterioration [17]. MICs of cefoxitin and imipenem were higher for the rough morphotype than for the smooth morphotype (p 0.022 and p 0.004, respectively, Mann–Whitney *U*-test). The cefoxitin-resistant strain of *M. bolletii* showed a rough morphotype (Table 1). It remains to be determined whether this difference directly reflects a loss of glycopeptidolipid production or, alternatively, results from target modifications associated with prolonged exposure of rough isolates to the antibiotic selective pressure during chronic infection.

The MBCs of cefoxitin and imipenem were determined in the exponential and stationary phases of growth (Table S2). The MBC_{50} values were found to be one or two dilutions higher than the MICs in exponentially growing bacteria (Table S2). Neither cefoxitin nor imipenem was bactericidal in the stationary phase (MBC >256 mg/L).

MICs of the two β-lactams were lower for 7H9c than for CAMHB ($p < 10^{-4}$, Mann–Whitney *U*-test), and the difference was more pronounced for imipenem than for cefoxitine (Table 2). The MIC ranges were narrower in 7H9c than in CAMHB. 7H9c is commonly used for the growth of mycobacteria, as it allows greater ease of handling and the use of more uniform inocula. However, this medium cannot be

TABLE 1. MICs (mg/L) of cefoxitin (FOX) and imipenem (IPM) determined in cation-adjusted Mueller–Hinton broth for 43 clinical isolates of *Mycobacterium abscessus* complex

Drug	Clinical isolates (no. of strains)	No. of strains with indicated MIC						MIC_{50}	MIC_{90}	R (%)
		≤4	8	16	32	64	128			
FOX	All (43)			*8*	28	6	**1**	32	64	2
	M. abscessus (15)			*4*	11			32	32	0
	M. bolletii (14)				10	3	**1**	32	64	7
	M. massiliense (14)			*4*	7	3		32	64	0
	Smooth (25)			*6*	18	1		32	32	0
	Rough (18)			*2*	10	5	**1**	32	64	6
IPM	All (43)	*1*	11	25	**5**	**1**		16	32	14
	M. abscessus (15)		6	7	**2**			16	32	13
	M. bolletii (14)		3	10		**1**		16	16	7
	M. massiliense (14)	*1*	2	8	**3**			16	32	21
	Smooth (25)	*1*	9	14	**1**			16	16	4
	Rough (18)		2	11	**4**	**1**		16	32	28

Italic and bold type indicate susceptible and resistant categories, respectively, of interpretive criteria for each antimicrobial agent according to the 2011 CLSI breakpoints [14].