Demonstration of Plasmid-Mediated Drug Resistance in *Mycobacterium abscessus*

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Plasmid-mediated kanamycin resistance was detected in a strain of *Mycobacterium abscessus* subsp. *bolletii* responsible for a nationwide epidemic of surgical infections in Brazil. The plasmid did not influence susceptibility to tobramycin, streptomycin, trimethoprim-sulfamethoxazole, clarithromycin, or ciprofloxacin. Plasmid-mediated drug resistance has not been described so far in mycobacteria.

Plasmid-mediated drug resistance is uncommon in mycobacteria. A 56,267-bp circular plasmid of the broad-host-range IncP-1 β subgroup was detected in a strain of *Mycobacterium abscessus* subsp. *bolletii* (formerly known as *Mycobacterium massiliense* [1] and *Mycobacterium abscessus* subsp. *massiliense* [2]) that was responsible for a nationwide outbreak of mycobacterial surgical infections in Brazil in 2004 to 2008 (3, 4). This plasmid, named pMAB01, contains a complete system for conjugative DNA transfer and two genetic load regions carrying antimicrobial resistance genes, including the Tn5393c streptomycin resistance (*str*) transposon and a typical class 1 integron with an incorporated gene cassette encoding an aminoglycoside 6'-N-acetyltransferase (*aac*[6']-Ib), the dihydropteroate synthase type 1 gene (*sul*I), and a conserved open reading frame (*orf*5) that carries a GCN5-like N-acetyltransferase (3). We investigated the contribution of this plasmid to antimicrobial drug resistance in *M. abscessus* and in *Escherichia coli*.

The MICs of streptomycin (STR), kanamycin (KAN), tobramycin (TOB), trimethoprim-sulfamethoxazole (SXT), clarithromycin (CLR), and ciprofloxacin (CIP) were evaluated against the prototype *M. abscessus* subsp. *bolletii* outbreak strain INCQS 00594, the INCQS 00594 strain cured of the plasmid after 10 *in vitro* passages in liquid medium, and two additional outbreak isolates, one harboring the pMAB01 plasmid and one lacking it (Fig. 1A). Two *E. coli* strains, BL21(DE3) and a spontaneous mutant of *E. coli* C600 (5) showing nalidixic acid resistance (C600Nal^r), and the same strains harboring the pMAB01 plasmid were tested (Fig. 1B). The experiments performed to introduce pMAB01 into *E. coli* were detailed in a previous publication (3). In brief, competent *E. coli* BL21(DE3) isolates were transformed by electroporation with the pMAB01 plasmid purified from the INCQS 00594 isolate. In addition, mating experiments were carried out to confirm the conjugative capacity of the pMAB01 plasmid. Conjugative transfer of pMAB01 from *M. abscessus* to *E. coli* BL21(DE3) occurred at a frequency of 5.24×10^{-8} transconjugants/recipient and from the *E. coli* BL21(DE3) transconjugant to another *E. coli* strain, C600Nal^r, at a frequency of 1.14×10^{-6} transconjugants/recipient (3). The *E. coli* C600Nal^r strain was used as recipient in mating experiments with two *E. coli* strains to simplify the screening of *E. coli* transconjugants on nalidixic acid plates.

The MICs were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute documents M24-A2 and M100-S23 (6, 7). *M. abscessus* subsp. *bolletii*

FIG 1 Isolates included in the study. (A) *M. abscessus* subsp. *bolletii* isolates obtained during the epidemic of surgical infections were typed by pulsed-field gel electrophoresis (PFGE-DraI). Lane 1, isolate INCQS 00594; lane 2, cured INCQS 00594 colony; lane 3, isolate CRM0019 obtained in 2006 in Rio de Janeiro, Brazil; lane 4, isolate IAL2697 obtained in 2008 in São Paulo, Brazil. The asterisks indicate the plasmid bands. (B) Plasmids from *E. coli* were extracted with a Qiagen plasmid minikit. Lane M, *E. coli* NCTC39 R861 (used as marker); lane 1, *E. coli* BL21(DE3); lane 2, *E. coli* BL21(DE3) transformed with pMAB01; lane 3, transconjugant *E. coli* BL21(DE3); lane 4, *E. coli* C600Nal^r; and lane 5, transconjugant *E. coli* C600Nal^r.

was cultivated in cation-adjusted Mueller-Hinton broth (Oxoid, Hampshire, United Kingdom) for 3 to 5 days at 30°C, and *E. coli* was cultivated on LB agar plates for 16 h at 37°C. Bacterial suspensions were prepared and adjusted to a 0.5 McFarland standard and diluted to achieve a final inoculum of approximately 5×10^5 CFU/ml. The following drug concentrations were tested: 0.5 to 64 μ g/ml (STR, KAN, and TOB), 0.12 to 16 μ g/ml (CLR and CIP), and 0.25/9.5 to 32/608 μ g/ml (SXT). *Staphylococcus aureus* ATCC 29213 was used as the control strain.

The MIC (μ g/ml) results are listed in Table 1. The plasmid

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TABLE 1 MICs of STR, KAN, TOB, SXT, CIP, and CLR against *M. abscessus* subsp. *bolletii* and *E. coli*

Species and strains	STR		KAN		TOB		SXT		CIP		CLR ^b	
	Presence of pMAB01		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)	
	Susceptibility ^a	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility	Susceptibility
<i>M. abscessus</i> subsp. <i>bolletii</i>												
INCQS 00594	+	>64	NA	R	32	R	>32/608	R	8	R	≤0.12	S
Cured INCQS 00594	–	64	NA	S	16	R	8/152	R	8	R	≤0.12	S
CRM0019 (2006)	–	>64	NA	S	16	R	8/152	R	16	R	≤0.12	S
IAL2697 (2008)	+	>64	NA	R	32	R	>32/608	R	8	R	≤0.12	S
<i>Escherichia coli</i>												
<i>E. coli</i> BL21(DE3)	–	4	NA	S	1	S	≤0.25/9.5	S	≤0.12	S	>16	NA
Transformed <i>E. coli</i> BL21(DE3)	+	>64	NA	R	64	R	0.5/19	S	≤0.12	S	>16	NA
Transconjugant <i>E. coli</i> BL21(DE3)	+	16	NA	I	16	R	≤0.25/9.5	S	≤0.12	S	>16	NA
<i>E. coli</i> C600Nal ^r	–	4	NA	S	1	S	≤0.25/9.5	S	0.25	S	16	NA
Transconjugant <i>E. coli</i> C600Nal ^r	+	>64	NA	R	16	R	≤0.25/9.5	S	0.5	S	16	NA

^a Interpretations of MIC results according to CLSI guidelines are in bold type. NA, not available; R, resistant; S, susceptible; I, intermediate.
^b CLR MIC was read at 3 to 5 days and did not include extended incubation up to 14 days for detection of macrolide induction.

contributed to the resistance of the *M. abscessus* subsp. *bolletii* outbreak strain to KAN. However, all of the tested mycobacterial isolates were resistant to other aminoglycosides, including STR and TOB, regardless of the presence or absence of the pMAB01 plasmid, suggesting that chromosomally encoded mechanisms may be responsible for resistance to these drugs in the outbreak strains. This is supported by the fact that for transformed and transconjugant *E. coli* BL21(DE3) and C600Nal^r, pMAB01 was associated with acquired resistance to all aminoglycosides tested, while no or 1 log₂ dilution variation was seen for STR and TOB among plasmid-positive and -negative *M. abscessus* subsp. *bolletii* strains. In addition, previous publications have confirmed that outbreak strains, whether harboring the pMAB01 plasmid or not, are fully susceptible to amikacin (AMI) (MIC = 4 μg/ml or 8 μg/ml) (8–10).

Acquired resistance to aminoglycosides in mycobacteria is primarily based on modification of the 30S subunit of the ribosome, caused by mutations in the 16S rRNA gene, *rrs*, or the *rpsL* gene, which encodes the S12 ribosomal protein (11). Mutations conferring resistance to 2-deoxystreptamine aminoglycosides in *M. abscessus* were identified at positions 1406, 1408, 1409, and 1491 in the *rrs* gene (12). In *Mycobacterium smegmatis*, changes in codons 43, 86, 88, and 91 of the S12 protein were identified in streptomycin-resistant mutants (13). These substitutions are not present in the *rrs* and *rpsL* genes from the INCQS 00594 strain (contigs 2 and 1, GenBank accession numbers AUVF01000002 and AUVF01000001, respectively). The existence of additional not-yet-identified mutations conferring aminoglycoside resistance in rapidly growing mycobacteria cannot be disregarded.

N-Acetylation and phosphorylation of a particular amino group are chemical modifications of aminoglycoside antibiotics that can result in decreased affinity of the drug for its target. The complete genome sequence of *M. abscessus* ATCC 19977 (GenBank accession number CU458896) was annotated with 5 putative aminoglycoside phosphotransferase genes and one aminoglycoside 2'-N-acetyltransferase gene (14). The role of these chromosomal enzymes in aminoglycoside resistance in mycobacteria has not been demonstrated (11). The plasmid pMAB01 carries an aminoglycoside 6'-N-acetyltransferase, a GCN5-related N-acetyltransferase (GNAT), and two *str* genes, encoding aminoglycoside-3'-O-phosphotransferase and aminoglycoside-6-O-phosphotransferase, both of which are required for high resistance to streptomycin (15). The results obtained here indicate that chromosomally and plasmid-encoded enzyme(s) contributed to KAN, STR, and TOB resistance in the *M. abscessus* outbreak strain, while in *E. coli*, the resistance was mediated solely by pMAB01-encoded enzymes.

Despite the fact that all of the tested mycobacterial strains were resistant to SXT, the MIC value of SXT was 4-fold higher in *M. abscessus* isolates harboring the plasmid than in those lacking it. The plasmid did not change the MIC of SXT in *E. coli* recipient strains, which remained susceptible after the acquisition of pMAB01, suggesting that the *sulI* gene could be nonfunctional in *E. coli*. *sulI* is a dihydropteroate synthase (DHPS) type 1 gene that catalyzes the formation of the immediate precursor of folic acid and is implicated in resistance to sulfonamide. The type 2 enzyme is stable, whereas type 1 DHPS loses its activity rapidly. This property could explain the lack of activity of the DHPS in *E. coli* harboring the pMAB01 plasmid.

The MICs of CLR and CIP against *M. abscessus* subsp. *bolletii* and *E. coli* were not affected by the presence or absence of pMAB01, suggesting that nonspecific resistance mechanisms, such as changes in cell wall permeability or the action of efflux pumps, were not induced by this plasmid.

In conclusion, genetic exchange, especially the acquisition of drug resistance promiscuous plasmids, could result in the emergence of specific mycobacterial strains with altered resistance profiles, which might be better adapted to cause human disease. The demonstration of plasmid-mediated resistance to KAN in an outbreak strain of *M. abscessus* suggests that the impact of resistance plasmids in environmental mycobacteria deserves further investigation.

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