ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2010, p. 5167–5172 Vol. 54, No. 12
0066-4804/10/$12.00 doi:10.1128/AAC.00610-10

Rv1218c, an ABC Transporter of *Mycobacterium tuberculosis* with Implications in Drug Discovery▽†

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Received 3 May 2010/Returned for modification 28 June 2010/Accepted 20 September 2010

Efflux systems are important in determining the efficacy of antibiotics used in the treatment of bacterial infections. In the last decade much attention has been paid to studying the efflux pumps of mycobacteria. New classes of compounds are under investigation for development into potential candidate drugs for the treatment of tuberculosis. Quite often, these have poor bactericidal activities but exhibit excellent target (biochemical) inhibition. Microarray studies conducted in our laboratories for deciphering the mode of action of experimental drugs revealed the presence of putative ABC transporters. Among these transporters, Rv1218c was chosen for studying its physiological relevance in mediating efflux in *Mycobacterium tuberculosis*. A Δ*Rv1218c* mutant of *M. tuberculosis* displayed a 4- to 8-fold increase in the inhibitory and bactericidal potency for different classes of compounds. The MICs and MBCs were reversed to wild-type values when the full-length *Rv1218c* gene was reintroduced into the Δ*Rv1218c* mutant on a multicopy plasmid. Most of the compound classes had significantly better bactericidal activity in the Δ*Rv1218c* mutant than in the wild-type H37Rv, suggesting the involvement of *Rv1218c* gene product in effluxing these compounds from *M. tuberculosis*. The implication of these findings on tuberculosis drug discovery is discussed.

Since its discovery in 1882 by Robert Koch, *Mycobacterium tuberculosis* has remained a clinician's and researcher's enigma until now. Tuberculosis caused by *M. tuberculosis* is responsible for approximately 2 million deaths each year (10) and remains a major health challenge. Various biological processes of the bacterium have been studied and elucidated in detail over the last couple of decades, but this has not aided in the development of new therapies to combat and eradicate this deadly pathogen.

Designing better drugs toward the treatment of tuberculosis is important in light of widespread emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* in certain parts of the world (5, 10, 12). The molecular mechanisms mediating drug resistance in many bacteria, including *M. tuberculosis*, have been described (8, 15, 17). Interestingly, horizontal gene transfer is absent in mycobacteria, as there are no plasmids reported from this bacterium (30). Additional mechanisms of drug resistance develop through mutation of the drug target, enzymatic modification of the target, and efflux of drugs from the bacterium. Recent research has shown that multidrug resistance of *M. tuberculosis* is associated with constitutive or inducible expression of efflux systems (7, 18). Therefore, understanding efflux mechanisms is becoming increasingly important in the area of tuberculosis drug discovery.

Efflux mechanisms that mediate bacterial resistance to known antibiotics have been well studied for several bacteria, including mycobacteria (4, 22, 23). Several putative efflux pumps in *M. tuberculosis* have been identified and characterized (1, 7, 9, 15). Overexpression of different efflux pump genes is found to be associated with resistance to multiple drugs in clinical isolates of *M. tuberculosis* (11, 14, 24, 25, 27). In addition, efflux pumps have been shown to be involved in virulence (26) oxidative stress responses, and growth (3).

Often in the tuberculosis drug discovery process, potent enzyme inhibition exhibited by project compounds does not translate into bacterial inhibition (MIC) or kill (MBC). One of the important reasons for this could be the cell wall architecture of mycobacteria, which may be impermeable to compounds. The lack of MIC could be further compounded by the presence of an array of efflux pumps, which are membrane proteins that export substrates across the cell membranes. These confer resistance to antibiotics in bacteria and provide low levels of intrinsic drug resistance (9). It has been observed that there is a vast overlap in substrate specificity among these pumps, which makes them redundant, and therefore it is difficult to specifically inhibit one pump in order to enhance the antimicrobial activity of compounds.

We have characterized one of the ABC transporters, Rv1218c of *M. tuberculosis*. Our studies suggest an important role for this gene product in mediating efflux to a wide variety of chemical classes, including novobiocins, biarylpiperazines, pyridines, bisanilinopyrimidines, pyrroles, and to a smaller extent pyrazolones.

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▽ Published ahead of print on 4 October 2010.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
M. tuberculosis H37Rv ATCC 27294	Virulent strain of *M. tuberculosis*	ATCC
pMV261	Mycobacterial expression vector	28
pBAN0192	936 bp of intact *Rv1218c* cloned into the PvuII-HindIII sites of pMV261	This study
pAZI0290	Derived from pGOAL19 (20) by deleting the *lacZ* gene as a BamHI-BamHI fragment	Lab stock
pBAN0366	Truncated *Rv1218c* cloned into pAZI0290	This study

MATERIALS AND METHODS

Bacterial strains, growth conditions, and chemicals. The strains and plasmids used in this study are listed in Table 1. *M. tuberculosis* strain H37Rv ATCC 27294, a mutant with a deletion in *Rv1218c* (the Δ*Rv1218c* mutant), and the Δ*Rv1218c* mutant complemented with plasmid pBAN0192 were grown in 250-ml roller bottles (Corning Inc., Corning, NY) as smooth cultures to mid-log phase (optical density at 600 nm [OD_{600}] = 0.5) and stored frozen as 0.5-ml aliquots in screw-cap cryo-vials (Corning) at −70°C. Representative vials from the frozen lot were thawed and plated for enumeration of viable counts. For subsequent experiments, seed lot vials were thawed, and the cells were diluted to the required CFU count per ml. The media used for growth of *M. tuberculosis* are Middlebrook 7H9 broth and 7H10 agar (Difco Laboratories, Detroit, MI) supplemented with 0.2% glycerol, 0.05% Tween 80, and 10% albumin-dextrose-catalase (ADC). Hygromycin B was purchased from Roche. *Taq* DNA polymerase and restriction enzymes (NcoI, BglII, PvuII, and HindIII) were purchased from Bangalore Genei (India). Kanamycin, tetracycline, novobiocin, ciprofloxacin, streptomycin, ethambutol, isoniazid (INH), ethidium bromide, verapamil, reserpine, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were purchased from Sigma. Oligonucleotides were synthesized by Sigma for this work.

DNA amplification by PCR. Screening of single-crossover (SCO) and double-crossover (DCO) recombinants in *M. tuberculosis* was done by PCR using *Taq* DNA polymerase. Single colonies were picked up from 7H10 agar plates, resuspended in 50 μl TE (10 mM Tris, 1 mM EDTA), and boiled for 10 min. A volume of 5 μl of the supernatant from the boiled samples was taken in 25 μl of PCR mixture. The details of PCR conditions and the sequences of primers used in this study will be made available upon request.

Plasmid constructs. The recombination substrate for creating a deletion in the *Rv1218c* gene of *M. tuberculosis* consisted of the mutant gene with flanking sequences cloned into a suicide vector, pAZI0290. The 375-bp deletion in the *Rv1218c* gene (from bp 1 to bp 375) was generated by overlapping PCR in such a manner that the deleted region was flanked by 800 bp on both 5′ and 3′ sides of the deletion. The 1,612 bp PCR-amplified DNA fragment was cloned into NcoI-BglII sites of the suicide vector pAZI0290 (Table 1) to obtain the KO construct pBAN0366. For making the complementation construct, the 936-bp *Rv1218c* gene was amplified from *M. tuberculosis* genomic DNA and cloned into PvuII-HindIII sites of the mycobacterial expression vector pMV261 (Table 1) to obtain pBAN0192. The sequence of the cloned fragments was confirmed by DNA sequencing (Microsynth). The recombinant plasmids were transformed into *M. tuberculosis* as described earlier (29).

Growth profile of the strains. The wild-type (WT) H37Rv strain, the Δ*Rv1218c* strain, and the complemented strain (Table 1) were grown in Middlebrook 7H9 broth as described above. Growth profiles were obtained by aliquoting 100-μl culture samples at 2- to 3-day intervals up to a period of 16 days. These samples were diluted and plated as described for enumeration of the CFU.

Determination of antibiotic susceptibility. To determine MICs of the standard drugs rifampin, isoniazid, streptomycin, ethambutol, ciprofloxacin, novobiocin, kanamycin, tetracycline, and ethidium bromide and of compounds under investigation on *M. tuberculosis*, the resazurin-based microplate assay was performed as described earlier (19). The effect of efflux inhibitors reserpine, verapamil, and CCCP on the MICs of these antibiotics was studied by incorporating the inhibitors at subinhibitory concentrations (0.25× MIC) into the *M. tuberculosis* cultures (WT and the Δ*Rv1218c* strain) in the assay (data not shown). Among these inhibitors, though reserpine had an MIC of >200 μg/ml, it was considered to be 200 μg/ml and 50 μg/ml was used as 0.25× MIC. The MICs of verapamil and CCCP were 200 μg/ml and 2 μg/ml, respectively, on these two strains, and 50 μg/ml and 0.5 μg/ml of each was used along with test compounds.

TABLE 2. MICs of pyrazolones on WT *M. tuberculosis* H37Rv in the presence of efflux inhibitors verapamil, reserpine, and CCCP

Compound	MIC, μg/ml (resistance ratio)[a]			
	7H9 broth	7H9 + verapamil	7H9 + reserpine	7H9 + CCCP
AZI-335	1	0.25 (4)	0.5 (2)	0.5 (2)
AZI-336	0.5	0.25 (2)	0.25 (2)	0.25 (2)
AZI-377	2	1 (2)	0.25 (8)	1 (2)
AZI-219	256	64 (4)	Not tested	128 (2)
AZI-410	64	16 (4)	64	16 (4)
AZI-408	64	16 (4)	32 (2)	16 (4)
AZI-352	>16	8 (>2)	8 (>2)	8 (>2)
INH	0.03	0.03	0.03	0.03

[a] MICs were performed as described in Materials and Methods by resazurin-based microplate assay. Verapamil and reserpine were used at 50-μg/ml and CCCP was added at 0.5-μg/ml concentrations. The resistance ratio (given in parentheses) is defined as $MIC_{7H9}/MIC_{7H9\ +\ respective\ inhibitor}$. Each reported value is from an average of at least two independent assay results.

Studies to assess bactericidal action. Assays were set up to assess the bactericidal action of representative compounds on the wild-type strain, the Δ*Rv1218c* strain, and the complemented strain. The protocol was as described earlier using the CFU-based evaluation (16) except that the assays were set up in a 96-well microplate format. Briefly, bacterial numbers were enumerated on 7H10 agar plates following exposure of these three strains to various concentrations of compounds for a period of 12 days. These plates were incubated for 21 to 28 days at 37°C, and colonies were scored.

RESULTS

In an attempt to understand the efflux pumps of *M. tuberculosis* and their involvement in mediating intrinsic drug resistance to project compounds in our target-based drug discovery program, we have profiled investigational drugs (which have potent enzyme inhibition but poor MICs), using efflux inhibitors such as verapamil, reserpine, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). During these screens, it was observed that compounds belonging to the class of pyrazolones had decreased MICs on *M. tuberculosis* in the presence of these efflux inhibitors (Table 2). Microarray data were generated for pyrazolones to understand their mode of action, which revealed a 4- to 6-fold hyperexpression of certain hypothetical ABC transporters besides a number of other genes. We focused our efforts on characterizing the role of one of these genes, *Rv1218c*, coding for a probable ABC transmembrane transport protein, on the physiology of *M. tuberculosis*.

Inactivation of *Rv1218c* in *M. tuberculosis*. *Rv1218c* of *M. tuberculosis* was inactivated by two-step homologous recombination as described previously (20). In order to ensure that no active *Rv1218c*-encoded protein was made, we deleted the initial 375 bp of the *Rv1218c* gene followed by an insertion of T, which rendered the remaining part of the gene out of frame. Since the deletion in the *Rv1218c* gene is unmarked, no polar effects on the transcription of the downstream genes are expected. Out of six colonies of single-crossover recombinants (SCO) screened, four colonies had the plasmid integrated in the proper locus as confirmed by PCR. One of the SCOs (no. 4) was grown in 7H9 broth in the absence of hygromycin for 3

TABLE 3. Susceptibility of WT *M. tuberculosis* H37Rv, the Δ*Rv1218c* mutant, and the complemented strain to experimental compounds

Chemical class	Compound no.	MIC, μg/ml (resistance ratio),[a] for:		
		Wild-type	Δ*Rv1218c* mutant	Δ*Rv1218c* mutant (pBAN0192)
Pyrazolones	AZI-335	1	1	Not tested
	AZI-336	0.5	0.5	Not tested
	AZI-371	128	128	Not tested
	AZI-377	2	1	Not tested
	AZI-219	256	128 (2)	Not tested
Pyridones	AZI-521	256	64 (4)	256
	AZI-510	256	128 (2)	Not tested
Biaryl-piperazines	AZI-524	256	128 (2)	256
	AZI-525	256	128 (2)	256
	AZI-526	64	32 (2)	64
	AZI-529	128	16 (8)	Not tested
Bisanilino-pyrimidines (BAPS)	AZI-224	32	8 (4)	Not tested
	AZI-284	32	8 (4)	Not tested
	AZI-167	128	16 (8)	Not tested
Pyrroles	AZI-530	0.12	0.03 (4)	0.12
	AZI-531	0.03	0.008 (4)	0.015
	AZI-532	0.03	0.008 (4)	0.015
	AZI-533	0.03	0.008 (4)	0.03
Novobiocin		2	1 (2)	4
Ethidium bromide		0.25	0.12 (2)	0.25
INH		0.03	0.03	0.03

[a] MICs were either determined by the resazurin-based microplate assay or read turbidometrically. The resistance ratio (given in parentheses) is defined as $MIC_{wild\text{-}type}/MIC_{\Delta Rv1218c\ mutant}$. Each reported value is from an average of at least two independent assay results.

weeks and then plated either on plain 7H9 plates or 7H9 plates containing 2% sucrose. The colonies growing in the presence of sucrose were screened for the loss of the plasmid by PCR amplification of *hyg*^{r} and *sacB* genes. Only 20 to 30 colonies grew on the sucrose plates. Out of these, nine colonies were picked and analyzed by PCR. Among these, six colonies had retained the wild-type copy of the gene on the chromosome, while three had the wild-type copy of *Rv1218c* replaced by the truncated copy, thus rendering the gene nonfunctional. The absence of the wild-type copy in one of the mutants (no. 4.2) was further confirmed by PCR and Southern blotting using appropriate primers (data not shown).

The wild-type H37Rv strain, the Δ*Rv1218c* strain, and the Δ*Rv1218c* mutant complemented with pBAN0192 grew normally in Middlebrook 7H9 broth. No obvious growth disadvantage was evident, as seen from the CFU (data not shown).

The Δ*Rv1218c* mutant is sensitive to new chemical classes. MIC assays set up with reference drugs streptomycin, isoniazid, rifampin, ethambutol, ciprofloxacin, tetracycline, and kanamycin revealed that there was no significant decrease in the MICs of these antibiotics for the Δ*Rv1218c* mutant (data not shown). The pyrazolone class also did not show any decrease in the MICs for the Δ*Rv1218c* mutant compared to that for the WT strain (Table 3). However, ethidium bromide, novobiocins, biarylpiperazines, bisanilopyrimidines (BAPS), pyrroles, and pyridones exhibited 4- to 8-fold decreases in their MICs for the Δ*Rv1218c* mutant (Table 3) compared to that of the WT strain.

Complementation of Δ*Rv1218c* with the *Rv1218c* gene restores the resistance to compounds. The undisrupted *Rv1218c* gene was cloned into the plasmid vector pMV261, and this plasmid was designated pBAN0192 (Table 1). *M. tuberculosis* strain H37Rv transformed with this plasmid (pBAN0192) carrying the wild-type copy of the *Rv1218c* gene did not show any increase in MICs, in contrast to the deletion mutant (data not shown). Furthermore, when pBAN0192 was introduced into the Δ*Rv1218c* mutant, it restored the MICs to the wild-type levels for those compounds that were affected in the Δ*Rv1218c* mutant (Table 3).

Efflux inhibitors decrease the MICs of pyrazolones on *M. tuberculosis*. MIC assays using compounds of the pyrazolone class were set up with efflux inhibitors verapamil, reserpine, and CCCP at subinhibitory concentrations (0.25× MIC) of the inhibitors on WT *M. tuberculosis* and the strain carrying the deletion mutation Δ*Rv1218c*. It was observed that the MICs decreased by 2- to 8-fold for most of these compounds (Table 2) in the WT strain in the presence of efflux inhibitors. In the Δ*Rv1218c* mutant the MICs of these compounds did not exhibit any decrease and one compound exhibited a 2-fold decrease (Table 3). However, in the Δ*Rv1218c* mutant the efflux inhibitors brought about a 2- to 16-fold decrease in the MIC values of the pyrazolones (Table 4).

The MIC of INH was not affected by the efflux inhibitors in both the strains. The pyrroles exhibited only a 2-fold drop in the MICs for the Δ*Rv1218c* mutant in the presence of efflux inhibitors (Table 4).

Cidality of representative compounds and reference drugs on *M. tuberculosis* (wild-type, Δ*Rv1218c* mutant, and complemented strain). Mycobacterial cultures treated with AZI-521,

TABLE 4. MICs of pyrazolones and pyrroles for the *M. tuberculosis* Δ*Rv1218c* mutant in the presence of efflux inhibitors verapamil, reserpine, and CCCP

Compound	Chemical class	MIC, μg/ml (resistance ratio)[a]			
		7H9 broth	7H9 + verapamil	7H9 + reserpine	7H9 + CCCP
AZI-335	Pyrazolones	1	0.5 (2)	0.5 (2)	0.5 (2)
AZI-371		128	16 (8)	32 (4)	64 (2)
AZI-219		128	18 (16)	128	128
AZI-530	Pyrroles	0.03	0.03	0.015 (2)	0.015 (2)
AZI-531		0.008	0.008	0.008	0.004 (2)
AZI-533		0.008	0.004 (2)	0.004 (2)	0.004 (2)
Novobiocin	Novobiocin	1	0.5 (2)	NT	NT
INH	INH	0.03	0.03	0.03	0.03

[a] MICs were performed as described in Materials and Methods by resazurin-based microplate assay. Verapamil and reserpine were used at 50-μg/ml and CCCP was added at 0.5-μg/ml concentrations. The resistance ratio (given in parentheses) is defined as $MIC_{7H9}/MIC_{7H9+\ respective\ inhibitor}$. Each reported value is from an average of at least two independent assay results. NT, not tested.

AZI-525, AZI-533, ethidium bromide, and isoniazid (INH) were plated for viable mycobacteria following drug exposure for 12 days. All the compounds tested on the Δ*Rv1218c* mutant with the exception of INH resulted in approximately a 1.0 to 2.5 $\log_{10}$ CFU/ml reduction compared to results for the wild-type or the complemented strain (Fig. 1). The bactericidal activity is clearly dose dependent for AZI-521, AZI-525, AZI-533, and ethidium bromide. The increased cell death seen with the compounds in the Δ*Rv1218c* strain was restored to values closer to those of the wild type in the complemented strain in

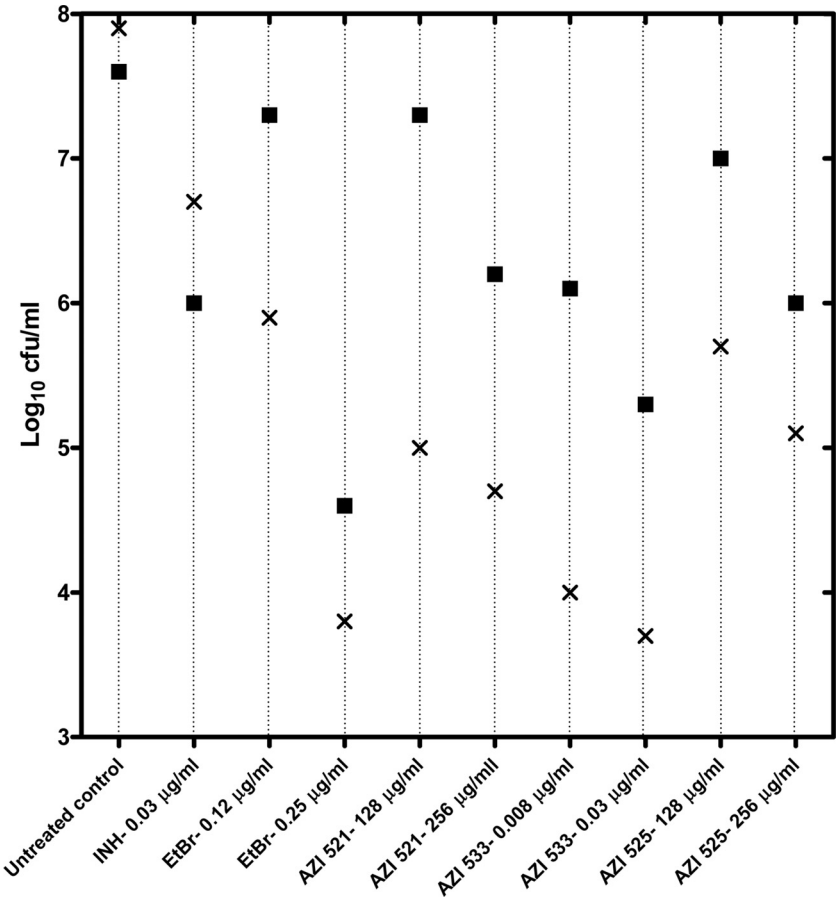

FIG. 1. Bactericidality of representative compounds on wild-type *M. tuberculosis* and the Δ*Rv1218c* mutant. Cultures were exposed to compounds at the concentrations indicated and processed as described in Materials and Methods. At the end of a 12-day exposure these cultures were plated for survivors, and colonies were counted after 4 weeks of incubation at 37°C. All compounds were tested at two concentrations: the higher concentration used for each compound is the MIC for the WT, while the lower concentration is the MIC for the Δ*Rv1218c* mutant. The MIC of INH was the same for both strains. The vertical (*y*) axis represents the number of bacteria surviving under each condition described on the horizontal (*x*) axis after compound exposure for 12 days. ■, wild type; ×, Δ*Rv1218c* mutant.

all the compounds tested (data not shown in the figure). The cell death observed with INH was to the same extent among all the three strains under investigation.

DISCUSSION

In this study, we have characterized a new major ABC transporter of *M. tuberculosis*, which is responsible for the efflux of a wide variety of substrates belonging to the diverse chemical classes of novobiocins, pyrazolones, biarylpiperazines, bisanilinopyrimidines, pyrroles, and pyridones. The MICs for many of these compounds decreased 4- to 8-fold for the Δ*Rv1218c* mutant, compared to that for the wild-type *M. tuberculosis* strain (Table 3). Further, these MICs were restored to that for the wild-type strain when the deletion mutant was complemented with an extrachromosomal copy of full-length *Rv1218c* on plasmid pBAN0192. These results strongly suggest the involvement of the pump encoded by *Rv1218c* in effluxing these compounds which could be potential substrates for this pump. The cidality assays further supported these findings. AZI-533, AZI-521, AZI-525, and ethidium bromide exhibited increased killing to different extents in the Δ*Rv1218c* mutant (Fig. 1), which were restored in the complemented strain to the extent observed in the wild-type strain.

Rv1218c was the first efflux pump identified for our studies from the microarray data that was available for the pyrazolone class of compounds. The MICs of the pyrazolone class of compounds did not show any significant decrease for the Δ*Rv1218c* mutant in comparison with the MICs for the wild type (Table 3). In earlier studies involving the WT *M. tuberculosis* strain, this class had shown a 2- to 8-fold decrease in MICs with efflux inhibitors (Table 2). Following these findings, MICs were tested on the Δ*Rv1218c* mutant with the same inhibitors and these compounds exhibited a 4- to 8-fold decrease in MICs. The most likely explanation for these findings is that there are other efflux systems which are operational in *M. tuberculosis* for this class of compounds and these efflux pumps could be inhibited by verapamil, reserpine, or CCCP. Experiments are in progress in our laboratory to study other ABC transporters in *M. tuberculosis.*

The pyrrole class of compounds exhibited an additional 2-fold decrease in MICs for the Δ*Rv1218c* mutant when assayed along with the efflux inhibitors, suggesting the presence of other efflux pumps in *M. tuberculosis* capable of transporting such compounds. The redundancy of efflux systems in bacteria is well known (3). In order to address this problem of functional redundancy, we can inactivate two or more ABC transporters simultaneously to achieve greater potency for a specific compound. Subsequent identification of an inhibitor which could inhibit multiple pumps is likely to enhance the potency of compounds.

The efflux pump encoded by *Rv1218c* belongs to the ABC-type transporter class and is believed to confer antibiotic resistance as its function (4). ABC transporters that give rise to clinically relevant multiple drug resistance in human or animal pathogens have not been described so far (22). Danilchanka et al. (7) have recently described a new multidrug efflux pump from *M. tuberculosis* belonging to the ABC type of transporters. These authors have shown that *Rv0194* codes for the pump which extrudes ampicillin, chloramphenicol, streptomycin, tetracycline, vancomycin, erythromycin, and novobiocin. Only the multiple-drug-resistance class of efflux pumps like the AcrAB of *Escherichia coli* has broad transport capacities. AcrAB belongs to the RND family of efflux pumps, which play a major role in multidrug resistance in Gram-negative bacteria (21). VcaM of *Vibrio cholerae* (13) and Rv0194 (7) of *M. tuberculosis* appear to be among the very few ABC transporters involved in resistance to structurally unrelated drugs. Colangeli et al. (6) have shown that in *M. tuberculosis*, *iniA* induction confers multidrug tolerance to at least INH and ethambutol. Their study suggests that *iniA* confers tolerance through a multidrug resistance pump-like mechanism and that *iniA* is essential for proper pump function.

Several efflux pumps have been shown to be hyperexpressed in a multidrug-resistant *M. tuberculosis* clinical isolate by reverse transcription (RT)-PCR (14). Microarray analysis has revealed the presence and probable involvement of 10 different efflux pumps belonging to the MFS, SMR, and ABC families in clinical isolates of multiple-drug-resistant *M. tuberculosis* strains during stress induced by common anti-tuberculosis drugs (11). These findings could further enhance the understanding of the *in vivo* behavior of these efflux pumps in patients being treated with antibiotics. Efflux systems are primarily responsible for fundamental cellular physiological processes and drug extrusion is a nonspecific, secondary role (21). Thus, physiological regulatory systems may determine the levels of drug resistance (26).

There is a dire need for novel methods to discover new drugs for the treatment of tuberculosis, especially the drug-resistant type, as the conventional processes are not able to keep pace with the speed at which resistance develops in this bacterial pathogen. Elucidation of the functions and substrate specificities of the efflux pumps of *M. tuberculosis*, especially those encoded by ABC transporter genes *Rv0194* and *Rv1218c*, should enable researchers to explore novel approaches to combat drug resistance with the existent drugs. Introduction of inhibitors of efflux pumps into the regimen as adjunctive therapy would be a novel strategy where the inhibitor can facilitate the enhanced uptake of specific antibiotics by inhibiting the pumps (3). Efflux pumps significantly contribute to drug resistance and thus to the failure of treatment in tuberculosis. This therapeutic failure stimulates the search for the development of efflux pump inhibitors as adjuvant therapies (2).

In this communication, we have reported the identification and characterization of a major ABC transporter, Rv1218c, which is involved in the resistance to several structurally unrelated classes of investigational drugs. By inactivating the *Rv1218c* gene to abrogate the function of the protein, we have shown the increase in the potency of these different classes of compounds on the *Rv1218c* deletion mutant of *M. tuberculosis.*

Our *in vitro* studies will be extended further to test the behavior of the Δ*Rv1218c* mutant along with the wild-type strain and the effect of treatment with experimental compounds in infected animals.

ACKNOWLEDGMENTS

We thank Sudha Ravishankar for the use of the microarray data and Anirban Ghosh and Disha Awasthy for help with some of the biology experiments.

Author contributions: M.B. conceptualized and designed research; S.K. carried out most of the experiments; U.S. designed the constructs for deletion mutants and guided their preparation. N.M. was involved in the generation of various constructs. S.S. and A.N. performed some crucial microbiology experiments. M.B. analyzed data and wrote the paper.

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