DECIPHERING THE ROLE OF PUTATIVE MYCOBACTERIAL EFFLUX PUMPS IN METAL STRESS, ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION

Abstract

Drug resistant *Mycobacterium tuberculosis* (*Mtb*) represents the leading cause of mortality globally. Mechanisms such as slow drug absorption along with cell wall impermeability and active efflux contribute significantly to the emergence of drug resistance. Efflux pumps actively expel a diverse array of drugs and toxins away from the target site, contributing as a significant factor in the failure of anti-tubercular drugs and treatment. Bacterial metal transporters regulate the metal homeostasis within the cellular compartments. Little is known about the dual activity of such transporters of *Mycobacterium sp* in antimicrobial resistance. In this study, we report the role of Rv3270, a P-type ATPase of *Mycobacterium tuberculosis*, CorA, a magnesium transporter of *Mycobacterium smegmatis* known for their involvement in combating Zn^{2+} and Mg^{2+} metal ion toxicity and Rv1250 a putative MFS transporter; in influencing the expulsion of different classes of drugs, enhancing the biofilm formation of hosts and increasing infectibility of *M. smegmatis* cells in murine macrophages. The genes were cloned in pBAD-18Cam and pMIND vector. The effect of ectopic expression of *rv3270* and *corA* in *E. coli* and *Mycobacterium smegmatis* on antibiotic resistance was elucidated in presence / absence of metal stress and inhibitors by determining minimum inhibitory concentrations using micro-broth dilution method. Further intracellular antibiotic accumulation assay was performed to determine the pump mediated efflux activity. Biofilm formation ability of the host cells expressing the pumps was determined through semi-quantitative assay using crystal violet staining and microscopy. The expression of transporters *in trans* decreased the susceptibility of the host cells for diverse array of antibiotics by several folds (2 – 32 fold) that was elucidated in presence and absence of metals, indicating its involvement in imparting an intrinsic drug resistance. Increased efflux of fluoroquinolones and beta lactams from cells expressing the transporters was observed as revealed by the lower level of the antibiotic accumulation in the host. In addition, expression of these pumps resulted in an increase in biofilm formation of the host cells inside by 2 – 4 fold. Reserpine and CCCP acted as inhibitors for Rv3270 and CorA; on the other hand, Zn^{2+} and Mg^{2+} positively influenced the efflux activity of the transporters respectively. Rv1250, a putative MFS transporter enhanced the resistance of the host cells by 2 – 32 fold. Increased efflux of EtBr, Norfloxacin and Bocillin FL from cells expressing *rv1250* was observed as revealed by the lower level of the antibiotic accumulation in the host. In addition, expression of *rv1250* resulted in an increase in biofilm formation by 2 to 4 fold. The pump also enhanced the infectibility of the host *M. smegmatis* cells expressing it in murine macrophages. From a clinical perspective, targeting these pumps or its critical residues may provide a novel therapeutic avenue to restore antibiotic efficacy, limit persistence, and counteract efflux-mediated resistance mechanisms in *Mycobacterium tuberculosis.*

Keywords: Mycobacteria, efflux pump, biofilm, macrophage, antimicrobial resistance, metal transporters