SUMMARY

This study was initiated to search for non-lactam compounds which can tightly bind to the active site Mtb BlaC, and thus render it ineffective. In the beginning of the work, it was clear that all the known crystal structures resembled each other whether or not they were co-crystallized with inhibitor. Interestingly, even for Apo protein, the crystals were obtained with ampicillin present in the crystallization drop. We wondered if there was some effect of this component during seeding of the crystals or there is only one conformation of BlaC, out of various accessible ones in solution, which has a tendency or ability to settle in a lattice which provides diffraction quality crystals? Anyway, using the crystal structure of Apo BlaC, we could find non-lactam molecules which can possibly (theoretically) fit inside and bind to the active site. Testing the lead compounds, we found four new molecules which other researchers can take forward as antibiotics against Mtb and other bugs, as such or modify them as per experimental outcome(s). One unexpected outcome of this work was the observation that solution shape of Apo BlaC was significantly larger than the crystal structures. Results described above confirm that Apo BlaC exists in open conformation, ready to accept the antibiotics in its "open" active site (**Fig.6**). The data from BlaC in presence of sulbactam and tazobactam concludes that binding of antibiotic closes the enzyme, which opens again after "spewing" hydrolyzed contents. This cycle possibly repeats, until BlaC encounters a non-hydrolyzable molecule which can bind to its "open" active site viz. Clavulanate or our molecules

which induces closure of mobile helix or the "lid" of the active site, but now remains closed as BlaC cannot hydrolyze them out.

This renders BlaC irreversibly inactive, locked in the closed conformation. Primary outcome of our work will be of immense interest to researchers committed to structure-based drug discovery. Considering the ability of *Mtb* to mutate residues to escape inhibition by clavulanic acid, our molecules open up new possibilities, if not provide a proof-of-concept for others to take forward to overcome the challenge of drug induced resistance in bugs.

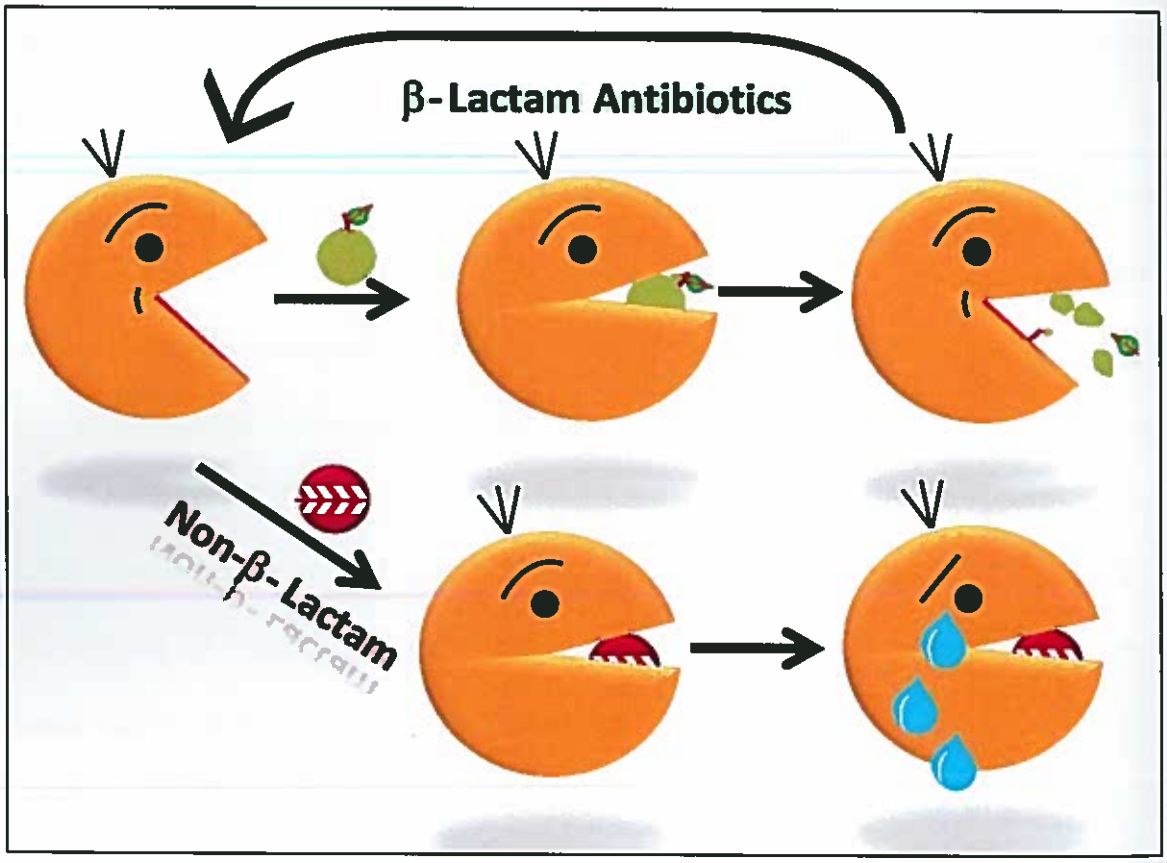

Fig. 6. Summarizing our findings that BlaC *exists in a shape which has its active site open, which closes on encountering antibiotic and again opens after hydrolyzing the molecule. In contrast, a non-hydrolyzable active site binding molecule renders enzyme in closed, inactive state.*