Title: Combating resistance to antibiotics by broadening the knowledge on molecular mechanisms behind resistance to inhibitor of cell wall synthesis
Acronym: COBRA
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Instrument: STREP

Summary:
The goal of Cobra was to better understand the various mechanisms of resistance to inhibitors of cell wall synthesis, in particular β-lactams, and to decipher recently appeared mechanisms of resistance. Our research have focus on various bacteria of clinical importance among which Gram-negative bacteria including members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*, as well as Gram-positive bacteria including *Staphylococcus aureus*, *Streptococcus pneumonia, Enterococcus faecalis,* and *Enterococcus faecium*. These bacteria are responsible for severe community-acquired and nosocomial infections. Most of the mechanisms of resistance explored in this STREP were either natural or acquired in the patient due to the selective pressure associated with the vast consumption of antibiotics. We have also characterize mutants selected *in vitro* to anticipate the emergence of new mechanisms of resistance. We have explored the mechanisms related to the modifications of essential targets, and associated critical factors connected with the expression of resistance, as well as the mechanisms of resistance due to enzymatic inactivation and decreased permeability. It resulted in the improve knowledge i) of the Structure-function studies of different penicillin target D,D-transpeptidases (PBPs); ii) of the enzymology of lipid-linked steps of peptidoglycan biosynthesis and identification of new enzymes among which some bypass the normal pathway of the peptidoglycan biosynthesis resulting in new peptidoglycan structures and resistance ; iii) of many new emerging beta-lactamases of class A,B,C and D which diversities , environments, structures and 3D structure/activity relationships were determined.

Problem:
Very few problems were encountered. More than 95% of the objectives were achieved. The only real problem was that some 3D structures could not be obtained due to intrinsic properties of the proteins which we tried to crystallize.

Aim:
Cobra considers the different problems that should be solved to address antibiotic resistance:
- Which structural features of different D,D-transpeptidase domains of PBPs involved in resistance to β-lactam antibiotics explain their low affinity for these antibiotics ?
- What is the role of associated critical factors and regulators involved in the expression of non β-lactamase-mediated resistance to cell wall inhibitors?
- Which are the catalytic mechanisms of the novel emerging, extended-spectrum β-lactamases of different classes with activity against β-lactams including third-generation cephalosporins and carbapenems, and how can they be understood in relation to enzyme structure?
- Which are the mechanisms involved in the regulation of the expression of different β-lactamase genes and what is the molecular basis for acquisition and mobility of new beta-lactamase as well as the role of impermeability in the expression of resistance?

Expected results:
- Understanding the role of those amino acid residues in PBPs that are essential for the expression of resistance and their contribution to the structure of the PBP D,D-transpeptidase domains.
- Understanding the biochemical role of the associated critical factors in the pathway of peptidoglycan synthesis, their structure, and their role in non β-lactamase-mediated

resistance; with respect to various regulators, understanding the mechanisms by which they interfere with the expression of resistance.
- Understanding the diversity of the structures of the beta-lactamases observed and studied, and understanding the role of the amino acid residues essential for their catalytic properties.
- Understanding the genetic environment of the β-lactamase genes and its contribution to resistance gene dissemination as well as the associated role of porins and efflux in expression of resistance.

Potential applications:
Our comprehensive approaches to determine the bacterial mechanisms of resistance to cell wall synthesis inhibitors should help clinical and industrial organizations involved in Bacteriology. More specifically, our work should allow to:
- document new beta-lactamases and the new strains with PBP alterations linked to resistance. (*Database to be released soon*)
- identify key targets thanks to a better comprehension of the peptidoglycan biosynthesis.
- address the role of non PBP factor in expression of beta-lactam resistance.
- optimize the mechanisms of transmission of new beta-lactamases.
- identify different Regulators for the expression of susceptibility and resistance.
- crystallize multiple enzymes and study their structure / function.

In fact, we can envisage that these results will now be exploitable for microbiology laboratories, healthcare services and pharmaceutical industry, on following actions:

- Detection of resistance in clinical laboratories.
- Identification of the phenotype associated with new resistance.
- New sequence-based molecular diagnostic tools.
- Implementation of dynamic assays for peptidoglycan cross-linking.
- Future setup of assays to screen for new inhibitors.
- Elucidation of different structures to improve drug design and search of inhibitors.
-Recognition of the antibiotics most suitable to select for the mechanisms studied and therefore gives guidance for their usage.
- Better knowledge of the emergence and dissemination of novel beta-lactamases and other mechanisms of resistance, the first step in their prevention.

Exploitation of the results obtained will likely be translated directly to the clinic as far as epidemiological surveys and recognition of new phenotypes are concerned. A longer delay will be necessary to translate our studies of some of our potential interesting targets into practice.

Project web-site: www.antibior.com
Key words: Resistance to antibiotics, cell wall synthesizing machinery, PBP, non-PBP factors and resistance , β-lactamases, catalytic mechanisms, structural studies, gene acquisition and mobility, Gram- positive and -negative organisms.

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