"ANALYSIS OF β-LACTAMASE PROTEINS THROUGH COMPUTATIONAL CHEMISTRY TECHNIQUES"

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María Elena Tejeda-Rosales
Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Zaragoza

Manuel Guillermo Sánchez-Tejeda
QFB. Universidad La Salle, México

Juan Francisco Sánchez-Tejeda
QFB. Universidad La Salle, México

Juan Francisco Sánchez-Ruiz
Ciencia y Estrategia, S.A. de C.V

ABSTRACT: Given the substantial implications of bacterial resistance for public health, it is imperative to comprehensively understand the mechanisms driving this resistance. Our aim is to undertake a taxonomical exploration of bacterial β-lactamases, with the goal of establishing a novel classification framework rooted in their molecular makeup, divergent from existing approaches. We developed a phylogenetic tree of clinically relevant bacterial β-lactamases utilizing their molecular sequences, subsequently generating a cladogram to unravel the distinctive traits of the various families and subfamilies. Objective: The aim of this study is threefold: firstly, to construct a phylogenetic tree of diverse β-lactamase enzymes sourced from clinically relevant bacteria using their molecular sequences; secondly, to elucidate the varied classifications observed in the resulting cladogram; and finally, to analyze the characteristics of the families and subfamilies of β-lactamase enzymes, which will be further explored in a multivariate analysis supported by computational chemistry.

INTRODUCTION

The potency of β-lactam antibiotics lies in their ability to hinder transpeptidases and carboxypeptidases activity by acylating a serine residue within the active site, notably in Penicillin-Binding Proteins (PBPs) (Fisher, 2005; Macheboeuf, 2006). Nonetheless, bacterial-produced enzymes called β-lactamases antagonize the efficacy of these antibiotics, fostering resistance against compounds like penicillin, cephalosporins, and carbapenems (Murray, 2017; Jawetz, 2014). With a surge in reports of antibiotic resistance in recent times (Wilke, 2005), there's an urgent call for phylogenetic analyses employing

contemporary molecular biology techniques (Attwood, 2002). These analyses are pivotal for unraveling the evolutionary path of β-lactamase proteins and their potential impact on clinically relevant microorganisms (Yamada, 2007).i

MATERIALS AND METHODS:

The search within the Protein Data Bank (PDB) for the target enzymes (8). Subsequently, the FASTA code corresponding to each enzyme was procured. Employing the CLUSTAL OMEGA server (9), we conducted alignment and multivariate analysis of the conglomerates to construct and ascertain the phylogenetic tree. Ultimately, a classification system was devised based on the findings derived from the phylogenetic tree.

RESULTS

The analysis of the cladogram obtained from CLUSTAL OMEGA validates the presence of three major families of β-lactamases, designated as I, II, and III. Family I comprise two distinct subfamilies, whereas family III encompasses four subfamilies (refer to Figure I).

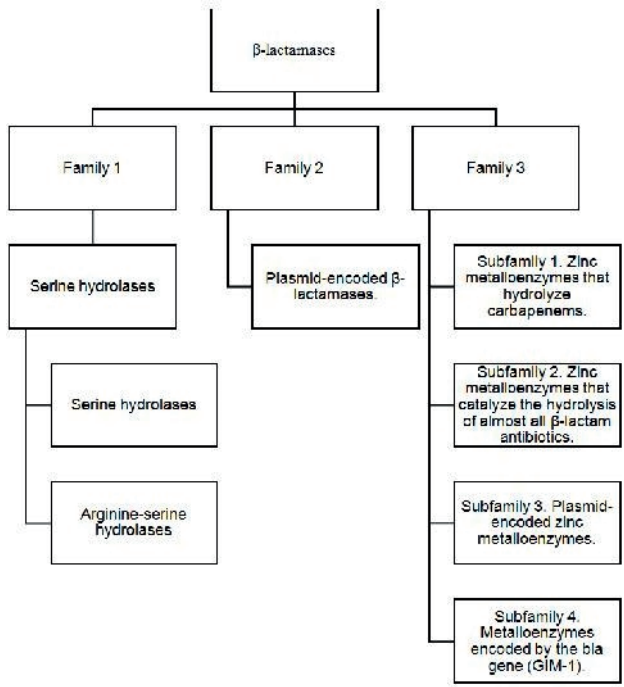

Figure 1. The classification based on the sequential alignment can be classified in 3 families. Family 1 has 2 subfamilies, while family 3 has 4 subfamilies

DISCUSSION

The cladogram uncovers the presence of three primary families of β-lactamases, labeled as Families 1, 2, and 3. Family 1 comprises two subfamilies: the first housing active serine hydrolases, characterized by the presence of serine within the active site, while the second subfamily hosts active β-lactamase hydrolases highly resilient to inhibitors like clavulanate, featuring arginine and serine at their active sites. Family 2 encompasses plasmid-encoded β-lactamases, whereas Family 3 comprises hydrolytic metalloenzymes, distinguished by metal cofactors within their active sites.

In contrast, the Ambler molecular classification, a prominent taxonomy of β-lactamases, delineates four families or classes: Class A (serine-penicillinases), Class B (metalloenzymes), Class C (serine-cephalosporinases), and Class D (serine-oxacillinases). Notably, the Ambler classification fails to discern molecular differences between β-lactamases of chromosomal or plasmid origin, a distinction evident in our study.

Regarding metalloenzymes or Class B, Ambler recognizes three subfamilies: Bl, B2, and B3. Subfamilies Bl and B3 comprise enzymes with broad-spectrum activity, while B2 primarily consists of Carbapenemases. However, our investigation identified four distinct subfamilies of metalloenzymes

Subfamily 1: Zinc metalloenzymes responsible for carbapenem hydrolysis.

Subfamily 2: Zinc metalloenzymes catalyzing the hydrolysis of nearly all β-lactam antibiotics.

Subfamily 3: Plasmid-encoded zinc metalloenzymes.

Subfamily 4: Metalloenzymes encoded by the bla gene (e.g., GIM-1).

This proposed classification provides a comprehensive overview of the diverse β-lactamase families and subfamilies identified in our study, as summarized in Figure 1.

CONCLUSION

The Ambler classification stands to benefit from expansion as it currently lacks a basis in phylogenetic analysis. Our study serves to complement and validate the Ambler classification, as demonstrated by our findings. This research represents an initial stride towards classifying β-lactamases utilizing protein molecular sequences. However, it's crucial to acknowledge that our analysis only encompasses the FASTA codes of clinically significant β-lactamases. To develop a more comprehensive classification, the inclusion of additional enzyme codes is imperative.

CONFLICT OF INTEREST STATEMENT

The authors declare no affiliations or involvement with any organization or entity having financial or non-financial interests in the subject matter discussed in this manuscript.

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