Hydrolytic Activity of OXA and CTX-M beta-Lactamases against beta-Lactamic Antibiotics

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Abstract. Beta-Lactamases OXA and CTX-M are highly disseminated and confer resistance to antibiotics. This project gathers, classifies and analyzes information from indexed articles and databases as UNIPROT, EMBL and PDB, in order to correlate molecular data with activity profile of beta-lactamases against beta-Lactamic antibiotics. Sequences of CTX-M and OXA enzymes are analyzed by ClustalW multiple alignment and distance trees are built by Neighbor – joining with default parameters and a Bootstrap of 1000, through MEGA 5.0.

Results from analysis were systematized with BLA.id system which documented 125 CTX-M, organized into four groups. Results show that CTX-M have punctual variations which change their hydrolytic activity profile. 310 OXAS grouped in 11 sets were analyzed and they show a high degree of conservation. Punctual changes in amino acids are correlated to changes in hydrolytic activity. BLA.id system fills a void in information about beta-Lactamases activity, which is disperse.

Keywords: beta-Lactamases OXA, beta-Lactamases CTX-M, antimicrobial sensitivity, Bioinformatics; Bla.id.

1 Introduction

Continuous rising in appearance and dissemination of antimicrobial drug-resistant microorganisms is a serious public health concern around the world. Inadequate use of antibiotics has applied selective pressure and this, added to diverse mechanisms of genetic transfer that bacteria can carry, contributes to multiresistant strains dissemination. [3]Design and development of new antibiotics have high costs, and as soon as a new antibiotic is created, microorganisms acquire new strategies to counteract its activity. [5] One of these strategies are beta-Lactamases, enzymes that hydrolyze amide bond of beta-Lactam ring. This makes the antibiotic loses its activity against cell wall synthesis [4] Currently, beta-Lactam antibiotics are the most viable alternative to treat bacterial infections and studying them is important and of a great relevance. It is especially important to focus on extended spectrum

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beta-Lactamases as CTX-M and OXA enzymes [9,10], as they are of high dissemination in Colombia. Biological data systematization, correlation between hydrolysis profiles and structural data will allow predicting their responses against different antibiotics. This is also important for rational selection of antibiotics and design of new and better drugs and also to contribute with WHO strategies [11, 12].

Ambler et al [6] classified beta-Lactamases in four classes (A, B, C, and D), in terms of the mechanisms of interaction enzyme-substrate and their primary structure, [1,2]. Class A (serine-penicillinases), class B (metaloenzymes), class C (serine-cephalosporinases) and class D (serine-oxacillinases) [8]. CTX-M beta-Lactamases belong to class A. They are defined by efficiently hydrolyze cefotaxime. OXA beta-Lactamases belong to class D and are defined by their capacity to hydrolyze more efficiently oxacillin and cloxacillin than conventional penicillins [13]

2 Materials and Methods

Initially we searched for general information on beta-Lactamases in databases as PDB (Protein Data BanK) and EMBL and in scientific articles regarding primary structure and hydrolysis profiles of beta-Lactamases. Analysis of this information allowed us to select beta-Lactamases for the study, considering the following issues: dissemination, resistance level, sequences and general information. We selected OXA and CTX-M beta-Lactamases to follow with next stages.

2.1 Information Searches, Analysis and Selection: OXA and CTX-M beta-Lactamases Sequences, Structures and Antibiotic Hydrolysis Profiles

Information collection was made using database http://www.lahey.org/studies, which keeps global information on beta-Lactamases, such as number of reported OXA and CTX-M; primary and tertiary structure. Information of activity profile of CTX-M and OXA against antibiotics was gathered from indexed scientific articles in databases as PubMed. Other specialized databases were consulted as: UNIPROT (Universal Protein Resource), PDB (Protein Data Bank), Drugbank and EMBL (Nucleotide Sequence Database Integration of data on each beta-Lactamase was made by SRS system. Information was classified as: type of beta-Lactamase, microorganism in which it is found, sequence, origin, tertiary structure and antibiotic resistance profile for each class of OXA and CTX-M.

2.2 Correlation of Sequence vs. Activity against Antibiotic Profile

Data were organized in a new database within BLA.id system. Correlation of beta-Lactamases sequences with their respective resistance profile was made through an unsupervised clustering of type sequences of different OXA and CTX-M, considering their differences patterns and hydrolytic activity profiles. Changes in sequences were analyzed by multiple alignment using ClustalW in Mega 5.0. All OXA and CTX-M sequences were entered and phylogenetic trees were done using Neighbor-joining and

maximum parsimony with 1000 replies each. The tree was validated with bootstrap method. Searches were made for the antibiotic susceptibility profile of each clade in the tree, taking into account the enzyme hydrolysis profile and MIC's against different antibiotics. Results were then interpreted according with CLSI 220430 guides.

3 Results

3.1 Search, Analysis and Selection of Information

Information search was focused on all OXA and CTX-M reported to date in different databases. With collected information we have a total of 256 OXA enzymes according Lahey Institute, from which, OXA-38, OXA-39, OXA-41, OXA-44, OXA-52, OXA-81, OXA-121 to OXA-127, OXA-135, OXA-140, OXA-151 to OXA-159, OXA-184, OXA-185, OXA-220 to OXA-222, OXA-226, OXA-227, OXA-232 to OXA-234, OXA-238, OXA-246, OXA-247, OXA-251 to OXA-255 have not yet been released but they were isolated, for this reason Lahey Institute classifies them as in assignation stage. It is worth noting that amino acids sequence of OXA-1 is the same as OXA-30 and amino acids sequences of OXA-24, OXA-40, OXA-46 and OXA-81 show not visible differences. (http://www.lahey.org/Studies/other.asp#table1)

There is a total 140 CTX-M, from which sequences of amino acids of CTX-M-14 and CTX-M-18; CTX-M-55 and CTX-M-57 are identical. There are reported as assigned: CTX-M-35, CTX-M-70, CTX-M-73, CTX-M-103, CTX-M-115, CTX-M-119, CTX-M-127, CTX-M-128, CTX-M-135, CTX-M-137, CTX-M-138, CTX-M-140 y CTX-M-141. CTX-M-118 is withdrawn. For this project we worked with information of 125 CTX-M in total.

Information was selected and organized according with the following criteria: group, class, family, variant, gene name, organism, UNIPROT, PUbMEd and GenBank references, isoelectric point, number of amino acids, sequence, sensitivity, origin and bibliography. Most difficult to find was information about sensitivity and isoelectric points, as it is not available for all OXA and CTX-M enzymes or there is not information available for the same antibiotic. Information was stored in BLA.id system (http://bioinf-servicios.ibun.unal.edu.co/BLA.id/), which allows the crosschecking of clinical and molecular data, for the identification of beta-Lactamases from bacteria that are resistant to beta-Lactamic antibiotics. This information system BLA_ID was implemented on a PowerEdge M605 Dell server by the Bioinformatics group at Biotechnology Institute of the National University of Colombia. Operative system is Linux SUSE 10, web server Apache 2.0; PHP version 4.3.4; MySQL version 4.0.18 and Perl version 5.8.3. It is available in http://bioinf.servicios.ibun.unal.edu.co/BLA.id/ [7].

3.2 Correlation of Sequence Data vs Antibiotic Resistance Profile

OXA. From information gathered about activity against antibiotics is concluded that on a general level it is few information about susceptibility profiles against different groups of antibiotics, except for carbapenems (imipenem and meropenem).

In all OXA's were identified structural motifs as 108STFK111, except for some changes in OXA-9 T109 S, OXA-62 F110Y, OXA-85 T109 S, OXA-50 F110Y, OXA-186 T109P and motif 185YGN187 typical of class D beta-Lactamases.

Distance tree groups OXA's in 11 clades or groups. (http://bioinf-servicios.ibun.unal.edu.co/BLA.id/arbolOXA.pdf). Clade 1 is largest with 79 OXA. Of them, OXA-51 confers resistance to oxacillin and OXA-68 and 86 to piperacillin. Regarding monobactamics, presence of OXA-68 and 86 confers resistance to aztreonam and there is scarce information about oximino-cephalosporins. Against carbapenems in general, there is information on meropenem and imipenem in 24 OXA's that produce profiles from susceptible to resistant. In the group of aminoglycosides, we found that OXA-194 confers to the microorganism resistance to kanamycin. OXA's belonging to clade 3, in general, confers resistance to carbapenems imipenem and meropenem, with exception of OXA-23 that confers resistance to imipenem. This group presents a change of conserved motif YGN, which is typical of class D beta-Lactamases by FGN, but as stated by some authors this does not modify imipenem hydrolysis.

12 OXA's belong to clade 6; OXA-60 hydrolyzes imipenem more efficiently than oxacillin, however, there is not enough information to correlate hydrolysis with susceptibility and its sequence. Clade 8 contains 23 OXA and they have the tetrad STFK and conserved motifs YGN, KTG which are typical of this class. In this clade OXA-16, OXA-14 and OXA-17 are the closest of the tree and they are very similar in sequence. These three OXA's do not confer resistance to carbapenems and they are present in Pseudomonas aeruginosa. OXA-17 does not have aspartate substitution in position 148, keeping glycine for which it does not confer significative resistance to ceftazidime, compared with OXA-14 and OXA-16. OXA's in this clade show changes in structural motifs KTG by KSG and in third conserved motif YGN by FGN. It seems this change affects NaCl inhibition. It is a great diversity in profiles and sequences between enzymes in this group.

Enzymes belonging to a same clade show few changes in amino acids, but, when comparing sequences between clades, there are several changes in amino acids. However, there is low information to correlate in accurate way susceptibility with sequences.

CTX-M. We used a total of 125 sequences out of 140 reported CTX-M. Enzymes were grouped in a dendrogram obtained by neighbor joining (http://bioinf-servicios.ibun.unal.edu.co/BLA.id/arbolCTXM.pdf). Alignment and dendogram show four groups. Comparison of these shows similitudes in amino acids sequences inside each group. There are highly conserver sequences as 73STSK76, 132SDN135 (with exception of CTX-M 81 which shows H135 by N) and 238KYGS241 (with exception of CTX-M 106 and CTX-M 107 238R por K), features associated with class A beta-Lactamases to which CTX-M belong.

Distance tree reveals four large clades. Clade 1 is formed by 46 CTX-M highly related. All enzymes show leucine 122 that seems to confer a higher resistance to ceftriaxone. CTX-M-11 shows proline at this position but there is not information

about hydrolysis or susceptibility against ceftriaxone,. For this reason it is not possible to affirm if this change affects resistance to this antibiotic.

CTX-M-62 and CTX-M52 show the change P170S, which contributes to efficiently hydrolyzing ceftazidime. Clade 2 has 21 enzymes with amino acid glycine 126, compared with other CTX-M that have serine in this position, its presence in microorganisms confer resistance to cefotaxime. Clade 3 is formed by 12 enzymes, of which CTX-M 40, CTX-M 63 and CTX-M 8 are the most distant from clade 4. CTX-M-40 confers to microorganism resistance to gentamicin and tobramycin but there is not enough information to correlate changes of sequence with those of susceptibility profile of microorganism that possess it. In this clade, leucine 122 is changed by phenylalanine, except for CTX-M 94, CTX-M 78, CTX-M 40, CTX-M 63 and CTX-M 8 which have the change alanine by glycine in position 123. Clade 4 contains 43 CTX-M with the most distant CTX-M-44, probed by alignment because it does not have amino acids R194 and N195. All enzymes in this clade have changed A65 by G and A70 by P. Some authors affirm that resistance to ceftriaxone is due to leucine 122, except for CTX-M-85 that has proline at this position, but it is not available information about microorganisms' susceptibility or hydrolysis of antibiotic to correlate this change with ceftriaxone resistance. CTX-M 93, 121,27, 98, 102, 105 and 16 change aspartate 242 by glycine, which increases hydrolytic activity against ceftazidime. CTX-M enzymes confer resistance to cefuroxime, cefotaxime and cefepime.

4 Conclusions

Available information on CTX-M and OXA enzymes is broad but scattered and it is mostly recorded in individual way. BLA.id system allows to systematize information in a dynamic way, trying to fill a void that currently exists as information about activity of beta-Lactamases is scattered and difficult to correlate. Multiple alignment generates information that allows to deduct punctual differences between different CTX-M and OXA. There is lack of information regarding their hydrolysis profile, or the susceptibility of microorganisms owing them; just some articles refer to enzymatic hydrolysis rate, which gives a higher reliability about enzymatic activity. In other publications minimal inhibitory concentration (MIC) is informed for a given microorganism which is less reliable information as activity against an antibiotic might be affected by other factors as simultaneous presence of other beta-Lactamase, efflux pumps, modifications in penicillin-binding proteins (PBP), among others. Given CTX-M and OXA diversity, their hydrolytic activity may be explained, in part, by amino acid sequence, but it is necessary to complement it with three-dimensional and functional information.

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