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Molecular docking analysis and determination of minimum inhibition concentration of VIM-38/R228S.

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Abstract

Objective: The aim of this study was to determine the effects of residues at 228th positions of VIM-38 on minimum inhibition concentration (MIC) and molecular docking analysis.
Materials and methods: blaVIM-38 gene was cloned into expression vector pET100/D-TOPO. Then pET100/D-TOPO-VIM-38 was used to generate the R228S mutation by site-directed mutagenesis. The mutant was transformed into E. coli Dh5α. Changes in the activity of mutation R228S was determined by E-test. Molecular docking analysis of the binding affinity of meropenem and imipenem with VIM-38 and VIM-38 R228S was performed using AutoDock Vina_1_1_2.
Results: According to E-test results, pET100/D-TOPO-VIM-38 and pET100/D-TOPO-VIM-38/ R228S have same MIC value for amoxicillin, ticarcillin/clavulanic acid, ampicillin and ticarcillin. It was determined decrease in pET100/D-TOPO-VIM-38/ R228S of MIC for aztreonam, amikacin and cefixine. There are 3-fold deference in MIC between pET100/D-TOPO-VIM-38 and pET100/D-TOPO-VIM-38/ R228S for cefoxitin. pET100/D-TOPO-VIM-38 has 0.5 µg/ml and pET100/D-TOPO-VIM-38/ R228S has 0.125 µg/m for imipenem, we observed 4-fold deference. Also, there is about 170-fold deference between pET100/D-TOPO-VIM-38 (16 µg/ml) and pET100/D-TOPO-VIM-38/ R228S (0.094 µg/ml) for meropenem. VIM-38 and VIM-38 R228S have very close to the negative low free energies of binding of the meropenem and imipenem.
Conclusion: These results showed that substitution (R228S) slightly have effect on enzyme activity.

Keywords: Docking, MIC, Mutation, VIM

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Introduction

Antibiotic resistance in the bacteria is a major problem worldwide. β-Lactam antibiotics are clinical significant in the treatment of bacterial infections, but resistance increasingly compromises their clinical use [1]. β-Lactamases are hydrolysed β-lactam antibiotics. β-Lactamases are the most important mechanism of resistance to β-lactam antibiotics. β-Lactamases are grouped into four classes: those in A, B, C and D. Class A, C and D enzymes making use of a catalytic serine to hydrolyse the β-lactam ring, while Class B comprises the metallo-β-lactamases (MBLs), which employ metal ions in catalysis [1]. MBLs or class B β-lactamases are zinc-dependent enzymes and they have broad hydrolytic activity against all β-lactams except aztreonam [1]. Genes encoding MBLs are located as cassettes in integrons which contribute to their dissemination and potential for expression [2]. To date, there are many type of MBLs identified such as the IMP, VIM, SPM, GIM, NDM, and SIM. The most commonly detected MBLs are IMP and VIM types in the worldwide [2]. Up to now, 46 VIM-type MBL variants have been described in total and each variant has one or several amino acid substitutions from each other (http://www.lahey.org/Studies/). Although VIM-type metallo beta lactamase have been identified in several Enterobacteriaceae [3]. VIM-1 was the first to be reported in 1999 in Italy, and then VIM-2 variant identified in France [4] and Italy [5]. VIM-5 was identified in Turkey in *Klebsiella pneumoniae*, *P. aeruginosa* and *Enterobacter cloacae* isolate [6-8]. VIM-38 has recently been identified in *P. aeruginosa* isolates in Turkey and, it differs from VIM-5 by a single substitution (Ala316Val) [9]. In a study on the enzymatic property of VIM-38, its steady-state kinetic analyses showed that VIM-38 hydrolysed all tested penicillins, cephalosporins and carbapenems [1].

The VIM-type enzymes belong to subclass B1 of molecular class B and VIM-type MBL has 3 different group (VIM-1, VIM-2 and VIM-7). VIM-38 belong to the VIM-1 cluster, and differ from VIM-1 by 6 residues (Ala130Lys, His224Leu, Glu225Ala, Ser228Arg and Lys291Thr, Ala316Val), respectively. VIM-38 contains His224Leu and Ser228Arg substitutions relative to VIM-1; these residues are positioned

on the L10 loop and are proposed to influence the substrate specificity of VIM variants [1,2,10-14].

It was aimed to determine the effects of residues at 228th positions of VIM-38 on minimum inhibition concentration (MIC) and molecular docking analysis in this study.

Material and Methods

Cloning experiments of blaVIM-38 gene

blaVIM-38 gene was detected by PCR. Cloning of the blaVIM-38 gene to expression vector pET100/D-TOPO was amplified using primers to obtain the whole gene sequence. The obtained PCR fragment was purified with a QIAquick column (Qiagen, Courtaboeuf, France) and cloned into the pET100/D-TOPO vector (Invitrogen Life Technologies, Saint Aubin, France). Recombinant plasmids were selected onto Mueller–Hinton agar (MH) plates containing ampicillin (50 mg/ml). The cloned DNA fragment inserted into one of the recombinant plasmids was sequenced by Macrogen. Sequencing results were analysed using alignment search tool, BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and the multiple sequence alignment program CLUSTALW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Site-directed mutagenesis of the R228S

Mutation was completed through blaVIM-38 allele which was cloned into the pET100/D-TOPO expression vector. Arginine amino acid position at 228 will be transformed Serine amino acids by directed mutations out of the pET100/D-TOPO-VIM-38. Primers were designed to generate the mutation. A single reaction mixture contained: 2 µl of plasmid DNA, 20 pM of each primer, 10 µl reaction buffer, 3 µl 25 mM $MgCl_2$, 200 µM of dNTPs and 1.5 U Pfu Polymerase (Promega, Madison, USA) in a final volume of 50 µl. PCR result was analysed on 1% agarose containing 0.5 µg/ml ethidium bromide, and subsequently visualized under UV light. PCR product was cleaned up by PCR-clean up kit (Promega, USA) and DpnI enzyme digestion were made. After digestion the samples were transformed into *E. coli* Dh5α. Plasmids were isolated and submitted to DNA sequence analysis. Sequencing results were analysed using alignment search tool, BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and the multiple sequence alignment program CLUSTALW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

E-test

The E-test was performed using E-test strips containing ticarcillin, tazobactam, aztreonam, cefixine, amoxicillin, amikacin, ampicillin, cefoxitin, ticarcillin/clavulanic acid, imipenem, meropenem and ertapenem according to the manufacturer's instructions in plates with MH agar. Plates were inoculated and incubated under the same conditions described for the agar dilution method, and MICs were determined.

Swiss modelling

Since the three-dimensional (3D) structure of VIM-38 and VIM-38 R228S are not known, the 3D structures of these β-lactamases were predicted by Swiss-Model [15-17]. Crystal structure of VIM-5 was used as a template.

Molecular docking

Molecular docking analysis of the modelled protein structures was carried out to identify if substitutions in the 228.amino acid of VIM-38 resulted in variations in their binding affinity to meropenem and imipenem. The structures of meropenem and imipenem were retrieved from the PubChem compound database. PyRx 0.8 was used for energy minimization calculations. Also, ligands and modelled proteins were converted PDBQT format with using PyRx 0.8. Grid box was cantered active site of proteins and enlarged the dimensions of the cubic search space to completely cover the site. Autodock Vina_1_1_2 was used for molecular docking calculations [18].

Results

blaVIM-38 gene was cloned to pET100/D-TOPO vector. Conversion of the arginine amino acid (at position 228) into serine amino acid was confirmed by sequencing analysis using pET100/D-TOPO _VIM-38 vectors. MIC values were determined by E-test method. MIC values are shown in Table 1.

Table 1. *MIC values of pET100/D-TOPO-VIM-38 and pET100/D-TOPO-VIM-38/ R228S.*

Antibiotics	pET100/D-TOPO-VIM-38 (µg /ml)	pET100/D-TOPO-VIM-38/ R228S (µg /ml)
Ticarcillin	3	3
Tazobactam	0.5	0.38
Azteonam	0.25	0.125
Cefixine	0.38	0.125
Amoxicillin	>256	>256
Amikacin	1.5	0.38
Ampicillin	>256	>256
Cefoxitin	6	2
Ticarcillin/clavulanic acid	>256	>256
Imipenem	0.5	0.125
Ertapenem	0.032	0.023
Meropenem	16	0.094

According to E-test results, amoxicillin, ticarcillin/clavulanic acid, ampicillin, and ticarcillin MIC values were found same for E. coli containing pET100/D-TOPO-VIM-38 and pET100/D-TOPO-VIM-38/ R228S. On the other hand, it was determined decrease in MIC for imipenem, meropenem,

tazobactam, aztreonam, amikacin, cefixine and cefoxitin. Also, R228S led to increase in MIC for ertapenem. pET100/D-TOPO-VIM-38 has 0.38 µg/ml while pET100/D-TOPO-VIM-38/ R228S has 0.125 µg/ml MIC for cefixime. pET100/D-TOPO-VIM-38 has 1.5 µg/ml and pET100/D-TOPO-VIM-38/ R228S has 0.38 µg/ml MIC for amikasin. pET100/D-TOPO-VIM-38 has 6 µg/ml and pET100/D-TOPO-VIM-38/ R228S has 2 µg/ml, there are 3-fold deference for cefoxitin. There are 4-fold deference MIC of imipenem for pET100/D-TOPO-VIM-38 0.5 µg/ml and pET100/D-TOPO-VIM-38/ R228S 0.125 µg/ml. Also, there is about 170-fold deference between pET100/D-TOPO-VIM-38 (16 µg/ml) and pET100/D-TOPO-VIM-38/ R228S (0.094 µg/ml) for meropenem (Table 1).

Analysis of binding affinity of meropenem and imipenem with VIM-38 and VIM-38 R228S was performed using AutoDock Vina_1_1_2. The negative low free energy of binding of docked complexes indicated high affinity of β-lactamase for these antibiotics [15]. The negative low free energies of binding of the meropenem and imipenem for VIM-38 and VIM-38 R228S were very close to each other (Table 2).

Table 2. *Molecular docking analysis results of VIM-38 and VIM-38/ R228S.*

VIM-38	Free Energy of Binding (kcal/mol)	VIM-38 R228S	Free Energy of Binding (kcal/mol)
Meropenem	-5.2 ± 0.01	Meropenem	-5.3 ± 0.33
İmipenem	-4.29 ± 0.31	İmipenem	-4.97 ± 0.34

Discussion

Creating the basic mechanisms of bacterial resistance to β-lactams are producing hydrolytic enzymes. These hydrolytic enzymes are called β-lactamases and they break the amide bond in the β-lactam ring of β-lactams [19,20]. So far more than 1000 β-lactamase has been reported [21]. These enzymes are encoded chromosomally or genes (that coding these enzymes) are located on mobile genetic elements such as transposons and plasmids. β-lactamases they show too many differences according to their functional, biochemical and similarity of amino acid sequences. According to Ambler molecular classification, β-lactamases can be divided into two super families structurally: serin (classes A, C and D) and metallo-β-lactamases (class B). Both serine β-lactamases and metallo-β-lactamases are able to hydrolyse the β-lactams, although catalytic mechanisms are different. The spread of MBLs among Gram-negative pathogens are a very serious problem [22]. More importantly, MBLs are carried with other resistance genes which restricted treatment options with the formation of multi-resistance [23]. Rapid spread of MBL are because of the mobile genetic elements such as plasmids, transposable elements and ISC relationship between MBL genes [22,23]. Spread of earned MBLs is a very crucial for infection control and the treatment of patients [22]. VIM-type of metallo- beta-lactamases have been reported to be 46 variants and they have one or more amino acid changes between these variants (http://www. lahey.org/Studies/). Each year, some of the new variants are defined in *Enterobacteriaceae*. Accumulated mutations on the gene take years and the amount of the accumulated mutations on a gene that gives information about the gene has a long history. Site-directed mutagenesis experiments on metallo-beta-lactamase conducted on the results are of great importance because occurring variants of increasing antibiotic concentrations (MICs) contain mutations selected on their ability to remain alive in the bacterial host. Therefore, experiments with purified recombinant proteins increase the understanding about effects of mutations that confer resistance [24].

At 228th position, VIM-2 has an arginine and VIM-1 has a serine. VIM-2 group enzymes interact directly with the substrate, whereas, VIM-1 group enzymes definitely too short to create any interaction with the substrate. This can be explained VIM-2 group has higher affinity for substrates [10]. Also, VIM-38 has arginine at position 228th.

In a study has been reported comparison of biochemical and biophysical properties of VIM-1, VIM-2, VIM-5 and VIM-38. The results showed that small differences in the kinetic parameters of the tested VIM variants [1]. In this study, substitution of Serine at the position 228 with Arginine, when compared with VIM-38 and VIM-38/R228S appears to give almost similar MIC values.

According to the results of molecular docking, the mutation at position 228 in VIM-38 showed that it did not affect binding affinity for imipenem and meropenem efficiently. To support this result, the binding energy can also be calculated with using different molecular docking programs. Because different molecular docking programmes can use different algorithms for calculating free binding energy [25]. Since the crystal structure of VIM-38 was not in Protein DataBank, the three-dimensional structure of proteins was obtained by using Swiss Model. In one study six different homology modeling programs; Modeller, SegMod/ENCAD, SWISS-MODEL, 3D-JIGSAW, nest, and Builder was compared. In this study it was found that three modeling programs, Modeller, nest, and SegMod/ ENCAD, perform better than the others [26]. 3D structures of proteins obtained with using different homology modelling programs can be used as receptors in docking programme. And docking results with these receptors can be compared. Differences between docking results and experimental data was found in many studies. It was showed that one of the reasons for differences between docking results and experimental data might be ignoring multiple binding in docking programmes [27]. In future studies, enzyme kinetics assays will be performed to determine precisely the effect of R228S mutation in VIM-38 on substrate affinity and catalytic efficiency.

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