**Table S1 Amino acid residues within 5Å in the active pocket**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Ala52 | Pro53 | Ala54 | **Ser55** | **Thr56** | **Tyr57** | **Lys58** | Val59 |
| Phe60 | Ser61 | Ala62 | Leu63 | Leu64 | Glu89 | Trp90 | Gln94 |
| Leu96 | Ser98 | Ala99 | Met100 | Ser101 | Ser102 | **Ser103** | **Ala104** |
| **Thr105** | Trp106 | Tyr107 | Phe108 | Gln109 | Lys110 | Asp112 | Leu120 |
| Tyr123 | Leu124 | Ile127 | Tyr129 | Ala138 | Tyr140 | Trp141 | Leu142 |
| Gln147 | Ile148 | Gln153 | Ser178 | Ile179 | Leu181 | Ser190 | Gly191 |
| **Lys192** | **Thr193** | **Gly194** | Thr195 | Ser196 | Ala204 | Gly205 | Trp206 |
| Phe207 | Val208 | Gly209 | Tyr220 | Ala231 | Gly232 | Ser233 | Ala235 |
| Ala236 |  |  |  |  |  |  |  |

Note: The overstriking indicated the AAs of active site andthe underlined represented the AAs involved in hydrogen bond forming.

**Table S2 The mutational sites with score of 1.0 predicted by SIFT**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| A11E | E16V | D18L | S19N | F21Y | S26E | G28S | S34E |
| N35K | R36E | K37D | T40E | R44K | K45E | A49E | F51V |
| **A52S** | **A54N** | **V59I** | **F60Y** | S68E | K73P | N74E | Q77Y |
| M78L | T79K | T83E | Q84E | Y87F | K88E | E89S | Q92E |
| F97E | S101E | S102N | A104V | **K110E** | R113E | I115V | E117Y |
| H119E | R121K | R122S | S128H | F134L | P137E | **A138E** | G144S |
| **Q147K** | N155E | M156L | Q169E | S170K | **S178A** | R180Y | E182S |
| E183S | N185E | G186E | R187Y | I188E | **S190Y** | V197E | E201K |
| L202D | H203V | **A204N** | **V208I** | A214E | E215D | F218Y | K228D |
| R229N | **A231T** | S233K | T234K | A239T | D244K | K245D | P250E |

Note: The overstriking mutational sites indicated AAs within 5Å of the active pocket but exclude AAs in the active sites and AAs forming hydrogen bond to drugs.

**Table S3 Scoring results by PolyPhen software**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Mutational site | Score | Mutational site | Score | Mutational site | | Score |
| A52S | 0.102 | A54N | 0.082 | | V59I | 0.048 |
| F60Y | 0.106 | K110E | 0.121 | | **A138E** | 0.038 |
| **Q147K** | 0.001 | S178A | 0.167 | | **S190Y** | 0.024 |
| A204N | 0.104 | V208I | 0.103 | | A231T | 0.138 |

Note: The overstriking mutational sites indicated the 3 mutational sites with the lowest score.

**Table S4 Determination of free sulfhydryl group in mutant protein**

|  |  |  |
| --- | --- | --- |
| Mutant protein | Absorbance value | C(Cysteine)(mM) |
| BlaR-CTD | -0.012728 | -0.005689509 |
| S19C/G24C | -0.0091324 | -0.004082249 |
| R50C/Q147C | -0.0050149 | -0.002241697 |
| S76C/L96C | 0.0028305 | 0.001265254 |
| S135C/S145C | -0.023145 | -0.010345984 |
| E183C/I188C | 0.063893 | 0.028560637 |

**Table S5 Acitivity identification of BlaR-CTD wild-type and mutant protein using HRP-AMP**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Protein | ND | Ampicillin | Penicillin G | Cefoperazone | Nafcillin |
| ND | 0.120 | 0.116 | 0.086 | 0.081 | 0.069 |
| BSA | 0.137 | 0.136 | 0.079 | 0.104 | 0.079 |
| BlaR-CTD | 2.207 | 0.134 | 0.114 | 0.115 | 0.205 |
| A138E | 2.489 | 0.171 | 0.140 | 0.121 | 0.398 |
| Q147K | 2.560 | 0.160 | 0.123 | 0.121 | 0.303 |
| I188K | 1.773 | 0.157 | 0.150 | 0.115 | 0.184 |
| S190Y | 1.265 | 0.130 | 0.105 | 0.111 | 0.149 |
| V197D | 2.319 | 0.364 | 0.160 | 0.229 | 0.321 |
| S19C/G24C | 2.472 | 0.347 | 0.340 | 0.187 | 0.413 |
| R50C/Q147C | 2.510 | 0.159 | 0.108 | 0.108 | 0.255 |
| S76C/L96C | 0.350 | 0.13 | 0.138 | 0.133 | 0.133 |
| S135C/S145C | 0.460 | 0.116 | 0.098 | 0.101 | 0.120 |
| E183C/I188C | 2.018 | 0.197 | 0.124 | 0.137 | 0.396 |

**Table S6 Binding sites of I188K/S19C/G24C to β-lactam antibiotics**

|  |  |  |
| --- | --- | --- |
| β-lactams | The AA sites involved in the formation of hydrogen bonds to β-lactams | Score |
| Pivampicillin | S55, M100, S103, K192, T193, T195 | 6.86↓ |
| Moxalactam | Y87, S55, K192, T195 | 4.93↑ |
| Cefapirin | K58, M100, S103, K192 | 4.78↓ |
| Cefalotin | Y87, S55, S103, T195 | 5.19↓ |
| Ceftazidime | E89, M100, S101, K192, T195 | 5.03↓ |
| Cloxacillin | S55, S103, T105, T195 | 3.89↓ |
| Carbenicillin | S55, S101, S103 | 4.37↓ |
| Cefoxitin | S55, S102, S103, T195 | 7.84↑ |
| Cefamandole | S55, S103, T105, T195 | 6.65↑ |
| Floxacillin | Y87, T105, G194 | 3.59↓ |
| Cefotaxime | S55, K58, S101, S103, T105, K192, T193 | 3.65↓ |
| Cefepime | E89, S55, S103, T105, T195 | 5.54↓ |
| Cefradine | S55, S103, T105, T195 | 6.59↓ |
| Cefalonium | S101, S103, K192, T193, G232, S233 | 4.72↓ |
| Cefquinome | S55, S103, T195 | 4.91↓ |
| Penicillin G | S55, S103, T105, T195 | 5.54↑ |
| Cefuroxime | S55, S101, S103, T195 | 6.65↑ |
| Dicloxacillin | S55, S103, T105, T195 | 4.71↓ |
| Cefaclor | S55, S103, T105, T195 | 6.31↑ |
| Cephradine | S55, S103, T105, T195 | 6.59↑ |
| Cefazolin | S55, S103, T105, T195 | 7.37↑ |
| Imipenem | S55, Y87, E89, T195, G232, S233 | 4.89↑ |
| Ampicillin | S55, S103, T105, G194, T195 | 4.86↑ |
| Sulbenicillin | Y87, S101, T195 | 3.43↓ |
| Ticarcillin | S55, S103, T105, T195 | 4.52↑ |
| Cefalexin | S55, E89, S103, T105 | 4.06↓ |
| Cefatriaxone | S55, E89, S103, T105 | 6.71↑ |
| Methicillin | K58, S103, K192, T193 | 4.81↑ |
| Piperacillin | S55, S103, T105, T195 | 5.01↑ |
| Azlocillin | S55, S103, T105, T195 | 5.31↑ |
| Cefadroxil | S55, S103, T105, T195 | 5.61↑ |
| Nafcillin | S55, S103 | 6.22↑ |
| Cephalosporin | S55, S103, T105, T195 | 7.31↑ |
| Amoxicillin | Y87, T105, T193 | 3.41↓ |
| Furbenicillin | S55, S103, T195 | 5.50↑ |
| Oxacillin | S55, S103, T105 | 3.14↓ |
| Aztreonam | S55, S103, T105, W141, T193, T195 | 4.53↑ |
| Ceftiofur | E89, S55, S103, T195 | 4.04↑ |
| Cefoperazone | S55, S103, T195 | 4.76↑ |
| Cefminox | S55, S101, S103, T193 | 4.55↑ |

Note: The arrow "↑" indicated higher score than wild-type proteins. The arrow "↓" indicated lower score than wild-type proteins.

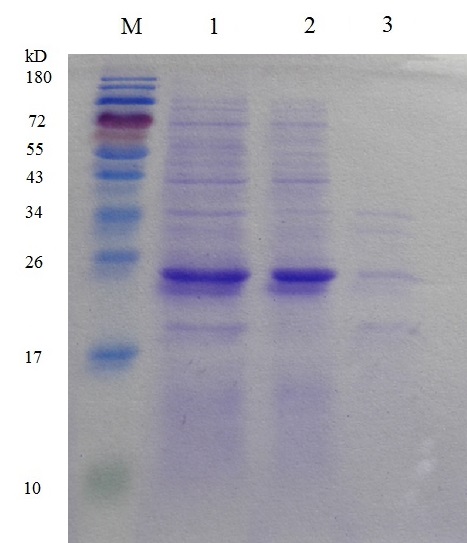
**Figure legend**

**Fig. S1 Expression of BlaR-CTD protein.** Lane 1 was the total cellular protein of *E. coli* BL21(DE3)/pET-28a(+)-BlaR-CTD after induction by IPTG; lane 2 was the soluble prtotein of the sonicated bacterial cell lysates; lane 3 was the insoluble proteins of the sonicated bacterial cell lysates. M, protein mass marker.

**Fig. S2 Standard curve of cysteine based on DTNB.** The abscissa is the concentration of cysteine and the ordinate is the absorbance at 412 nm.

**Fig. S3 The DNA and amino acid sequences of recombinant wildtype BlaR-CTD protein.** The recombinant protein included BlaR-CTD protein (amino acid number 1 to 254) and an N-terminal extension peptide containing His tag from pET28a vector (amino acid number -34 to -1).

**Fig.S1**



**Fig.S2**

**Fig.S3**

**3**